# Encapsulation & Immunoprotective Strategies of Islet Cells

December 6-7, 2001 • Washington, DC

Workshop Proceedings Report

**Sponsored by**

Juvenile Diabetes Research Foundation International
National Aeronautics and Space Administration
National Institute of Diabetes and Digestive and Kidney Diseases
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RTI – Center for Technology Applications
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# 1. Introduction

For nearly 20 years, research on encapsulating transplanted islet cells as a means of preventing rejection by the body's immune system has seen little progress. However, with isolated reports of success in a few experiments, the promise remains.

On December 6-7, 2001, more than 60 leading scientists from eight countries and other key individuals participated in a workshop to review the current state of encapsulation technology and to develop a strategy for the best use of a $2 million appropriation that the National Aeronautics and Space Administration will be directing toward research in this area.

Held near Washington, DC, the workshop included presentations from scientists on the latest technology, advances, and results in the field of encapsulation. Sessions were divided into four key areas: encapsulation and immunobarriers, industry perspectives, immunoprotective and anti-inflammatory therapies, and gene therapy for islet immunoprotection.

After the presentations and group discussions on Dec. 6, 2001, a smaller working group of senior and leading investigators met on Dec. 7, 2001 to identify important research issues that should become priorities for future funding. The group generally agreed that there are two high-priority issues: the need for successful large animal studies for further evidence and ultimate validation, and standardization of capsule materials and implantation procedures. Within that framework, the group drafted statements identifying eight specific research objectives, which are included in Section 6 of this report.

The workshop was sponsored by the following organizations:

- Juvenile Diabetes Research Foundation International
- National Aeronautics and Space Administration
- National Institute of Diabetes and Digestive and Kidney Diseases
- National Center for Research Resources

Research Triangle Institute organized the conference. The following individuals served on a planning committee that developed the conference and its goals:

- Stephen Davison, PhD, National Aeronautics and Space Administration
- Tom Eggerman, MD, PhD, National Institute of Diabetes and Digestive and Kidney Diseases, Islet Transplantation Program
- Daniel Jang, PhD, Juvenile Diabetes Research Foundation International
- David Klonoff, MD, FACP, University of California at San Francisco, Mills-Peninsula Health Services
- Ray Rajotte, PhD, University of Alberta, Surgical-Medical Research Institute
- Bernard Thorens, PhD, University of Lausanne, Institute of Pharmacology & Toxicology
- Gordon Weir, MD, Joslin Diabetes Center
- Dan Winfield, MS, RTI, Center for Technology Applications

## 1.1 Welcome and Introductory Remarks

*Charles Queenan III*
*Chair of Research*
*Juvenile Diabetes Research Foundation International*

As Chair of Research for Juvenile Diabetes Research Foundation International (JDRF), a major sponsor of the conference, Mr. Queenan welcomed the more than 60 participants from eight countries, and outlined goals for the two-day workshop:

- review what is known, and what is not known, about the potential use of islet cells to develop a successful artificial pancreas, as a cure for diabetes
- identify specific research and technical needs, with an emphasis on current priorities for new research
- stimulate new and meaningful research that can contribute toward finding a cure

He noted that he is the father of a teenager with diabetes, and added that the researcher's zeal for finding a cure often rivals that of a young patient's parents. The necessary research needed to help find a cure will be accomplished quicker and more effectively through a dynamic sharing of ideas and information. The sharing of ideas is the guiding principle behind the workshop.

Initial research into developing an artificial pancreas offered a promising pathway toward finding a cure, and consequently encouraged his family and many others with diabetic family members to believe such a cure may be achieved within only a few years, despite cautions from scientists and others. His expectations for when a cure may become available have since been tempered, as the complexity of the problem has become more obvious.

The development of an effective artificial pancreas will require careful and pains-taking research into a range of scientific issues and challenges. The search will be international in scope, and must involve close collaboration between both academic and industry medical entities, working in partnership with other affected parties.

The mission of JDRF is to help find a cure for diabetes. In doing so, JDRF hopes to facilitate the international partnership that will be needed, working closely with many groups – individuals who suffer from diabetes and their families, research scientists, donors, foundations, and government health agencies.

## 1.2 The Promise and Challenges of the Bioartificial Pancreas

*David C. Klonoff, MD*
*Mills-Peninsula Health Services, San Mateo, CA*
*University of California at San Francisco*
*Editor-in-Chief,* Diabetes Technology & Therapeutics

A bioartificial pancreas is a device that substitutes for an endocrine pancreas, that contains synthetic materials and functional islet cells encapsulated within a semipermeable membrane to protect them from immunological rejection. The current status of the bioartificial pancreas is first that the results are good in rodents, limited in large animals, and absent in humans, and second that better immunoisolation of islet cells is required.

The components of a bioartificial pancreas include islet cells, which are covered by a synthetic material and a barrier, which prevents contact with the host immune system. Pores within the membrane permit exchange of nutrients, glucose, and insulin with the host, but exclude immunoglobulins, complement, and white blood cells. The device is implanted into a well-perfused site.

The potential safe and effective cures for diabetes include islet cell transplantation, the artificial pancreas, and the bioartificial pancreas. Only the bioartificial pancreas provides both the safety of avoiding immunosuppressant drugs and the effectiveness of measuring blood glucose as accurately as only living islet cells can.

Successful development of a bioartificial pancreas will require four successful steps: 1) obtaining an adequate supply of tissue; 2) maintenance of cell viability; 3) protection of islet cells from immune rejection; and 4) practical implantation.

1) Three potential sources of islet cell tissue are currently under investigation. These include human or allogenic cells, porcine or xenogenic cells, and engineered cells. Human islet cells would be the least immunogenic type of cells. Unfortunately, there is a limited supply of donors and these cells have a limited secretory capacity and life span. Porcine islet cells are much more available because of an unlimited supply of donors, and the insulin sequence of a pig differs from that of human insulin by only a single amino acid. Unfortunately, porcine islets are extremely immunogenic.

Engineered islet cells are now being harvested from immortalized cells, insulinoma tumor cell lines, expansion and differentiation of stem cells, and from neogenesis of pancreatic duct cells. The source with the greatest potential for unlimited and non-immunogenic cells is some type of engineered islet cells.

2) Maintenance of cell viability is another important step needed to develop a successful bioartificial pancreas. The implanted islet cells are handled and then placed in an environment without natural circulation. The fabrication process must not damage the cells. An adequate supply of oxygen and other nutrients is necessary for successful implantation. In order to increase the local microcirculation the use of local vascular growth factors has been proposed. VEGF (vascular endothelial growth factor), FGF (fibroblast growth factor), and prolactin have all been proposed or tested for increasing the local microcirculation to the implanted bioartificial pancreas.

3) Regarding immune rejection, four types of fabrication architecture have been proposed for protecting islet cells – a vascular shunt, hollow fibers, small capsules, and thin flat sheets. The vascular shunt involves direct contact between the bloodstream and the chamber containing the islet cells, but the other three types of islet cell architecture involve indirect contact between the bloodstream and the implanted cells. Microspheres and thin flat sheets offer the greatest advantages for maintaining cell viability without causing complications associated with the implantation process. An ideal architecture for implantation without immunogeneticity would combine neovascularization around the implant without fibrosis, which is a late sequel of inflammation.

4) Practical implantation is a desirable feature of a bioartificial pancreas. Retrievable or biodegradable implants would eliminate unwanted long-term exposure to hardware. First-generation 0.7 mm microspheres required a 180 ml volume for implantation.

A bioartificial pancreas might be hampered by a delay in sensing fluctuations in blood glucose levels. Whereas a natural pancreas is exposed to fluctuations in blood glucose in real time, for an ectopically located bioartificial pancreas, depending on its implantation site, exposure to such fluctuations may lag by several minutes, because of site-specific differences in the glycemic responses to food and insulin. If the bioartificial pancreas is measuring interstitial fluid glucose, rather than blood glucose, then an additional lag in glycemic fluctuations may be overlaid on a site-specific lag, leading to further delays in initiation and cessation of insulin production in response to rising or falling blood glucose levels. A fibrotic capsule will form around certain implanted devices, which will further increase the lag between blood and interstitial fluid.

Desirable surgical features include minimal invasiveness, volume, obtrusiveness, and risk. A peritoneal implantation site is more physiologic for insulin action, but more invasive.

In conclusion, development of a bioartificial pancreas will require: 1) an unlimited supply of islet cells; 2) the avoidance of immunosuppressant drugs; 3) further advances in microencapsulation and immunoisolation of islet cells; and 4) U.S. Food and Drug Administration approval of each candidate technology as being safe and effective.

## 1.3 Challenges to Advancing the Bioarticifical Pancreas to the Clinic

*Steven Bauer, PhD*
*U.S. Food and Drug Administration*
*Division of Cellular and Gene Therapies*

Development of a bioartificial pancreas will involve two centers at the U.S. Food and Drug Administration (FDA). FDA's Center for Biologics Evaluation and Research would be the lead center, while the Center for Devices and Radiological Health would provide a review and consulting role. FDA uses an interdisciplinary, team-approach method to monitoring the progress of a new medical technology.

A number of regulatory concerns are common to the development of all new drugs and medical devices. FDA works to assure that new products are safe, effective, and pure; and that the identity is well-established and that potency is adequate. FDA regulates both the product and the process involved. Thus, regulation oversees quality control of the product and intermediates, and reproducibility of lots.

An investigational new drug (IND) application is managed by an FDA team that includes a product reviewer, and pharmacological/toxicological reviewer, and clinical reviewer, as well as consultant reviewers. Throughout clinical trials (phase I through phase III trials), the process requires annual reports, amendments, and adverse event reports to the FDA team. After a product is approved for licensing, FDA conducts post-approval surveillance of the product, including adverse reaction monitoring.

In March 2000, FDA's Biological Response Modifiers Advisory Committee discussed issues related to the use of human pancreatic islets for the treatment of diabetes, including product development issues relating to the procurement, processing and characterization of islets, preclinical animal models for islets and a brief clinical perspective. A summary of the advisory committee's findings is available through the FDA Web site at: http://www.fda.gov/cber/advisory/brm/brm0300.htm.

A number of issues involving development of a bioartificial pancreas and related products are central to FDA's consideration. These include a product's safety, toxicity, efficacy of cellular and device components, manufacturing consistency, and shipping and stability considerations. Other issues could involve the use of animal islets, use of stem cells, and a variety of device-related considerations.

For products such as a bioartificial pancreas, FDA involvement is available and would be helpful during product development and is required before conduct of clinical trials. General information about the regulatory procedure is available through FDA's Web site at http://www.fda.gov/cber, a library of fax advisories available at 888-223-7329, or through the center's Office of Communication, Training and Manufacturer's Assistance at 301-827-2000.

# 2. Encapsulation and Immunobarriers

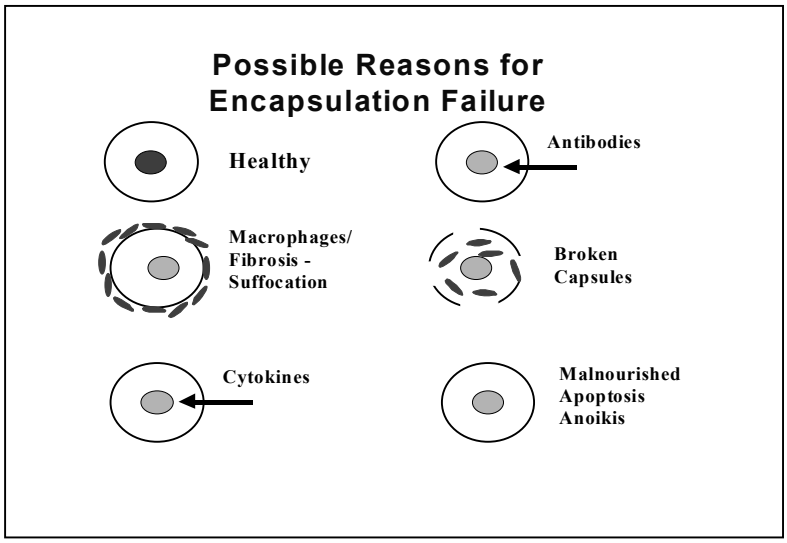
A session chaired by Gordon C. Weir, MD, of Joslin Diabetes Center, examined issues involving encapsulation and immunobarriers. Topics included an overview of encapsulation problems, oxygen requirements for islets, microcapsule chemistry, the search for a more perfect immunobarrier system, and the use of PEG to improve biocapatibility and permselectivity.

## 2.1 Encapsulation of Islets: How Difficult Are the Problems?

*Gordon C. Weir, MD*
*Joslin Diabetes Center*

The concept of protecting islet cells from immune destruction with an immunobarrier membrane is more than 30 years old. Alginate microcapsules containing islets were first described by Lim and Sun in 1980,[1] but in spite of the promise of the technique, convincing evidence of success in either non-human primates or humans has not emerged.

Various claims of success in humans, nonhuman primates and dogs have not been accepted as convincing by the scientific community. There are a variety of studies showing that immunobarrier membranes can protect islets in mice from autoimmunity, allorejection and xenorejection for over a year without immunosuppression. Very few studies with long term success in rats have been reported. Encouraging reports of studies in larger animals are even more scant.




Encouraging results from encapsulation development in mice include the fact that a biocompatible alginate for encapsulation is possible; traditional permselectivity is not needed for protection against allorejection and autoimmunity; islets in capsules can function effectively for over a year; and insulin release kinetics may be adequate. However, encapsulated syngeneic and allogeneic islets do not seem to do well in rats, and allografts are not yet working well in diabetic monkeys. Possible reasons for failure of encapsulated islets include suffocation, antibodies, cytokines, broken capsules, and malnurishment, apoptosis or anoikis.

Approaches that also rely on diffusion other than gel microcapsules include macrocapsules/beads and containers such as hollow fibers or planar sheets. For these, there are serious concerns about packing density, which could make surgical implantation impractical, and the kinetics of insulin release, which while adequate for rodents may not suffice for the meals, fasting and exercise of humans.

[1] Lim F, Sun AM. Microencapsulated islets as bioartificial endocrine pancreas. *Science* 1980 210:908-10.

For all the approaches, success in large animal studies remains elusive with the exception of the vascularized bioartificial pancreas in which the long term survival of adult porcine islets in dogs is convincing.[2] However, vascularized devices have their own separate drawbacks which may preclude their clinical use.

One can take the position that the approach of immunobarrier protection continues to have promise, but the field needs to find innovative and focused ways to determine whether clinical application can be successful. For this reason, challenging questions must be asked.

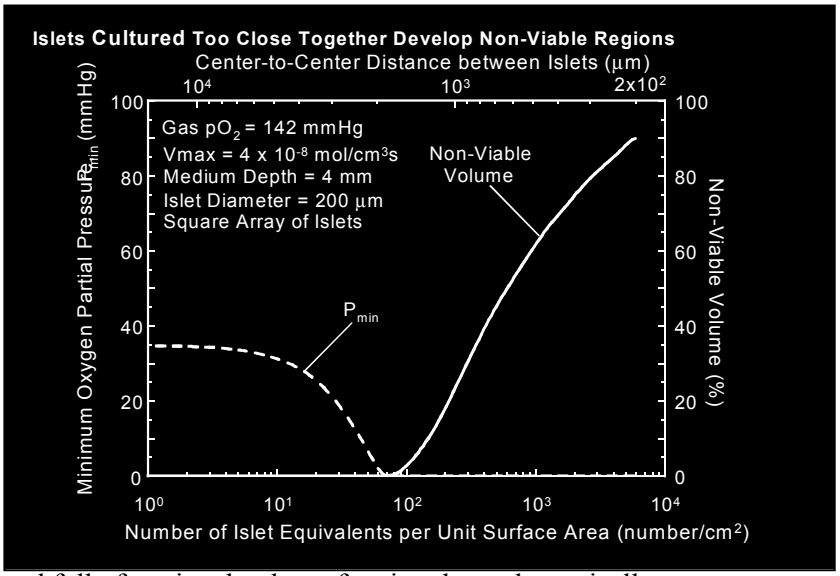
General questions about immunobarrier protection include the following:

- What is required for protection against allo-, auto- and xenoreactivity?
- How long can encapsulated islets function?
- What insulin release kinetics are required?
- Can a subcutaneous site be successful?
- What are the strengths and weaknesses of different animal models?
- What preclinical success should be required to justify human trials?
- If allografts are attempted in humans, how much beta cell mass will be needed? Will islets from two or more pancreases be needed?
- If success with isolated islets can not be achieved, could this technology be used to protect insulin-producing cells derived from stem cells or cell lines?

## 2.2 Oxygen Requirements of Islets

*Clark K. Colton, PhD*
*Massachusetts Institute of Technology*

Oxygen supply limitations can have deleterious effects on viability and functionality of encapsulated islets. This can be a contributing or primary cause for poor performance, or for failure of an implant.




Theoretical prediction based on oxygen diffusion and consumption models agree with experimental data for (1) size of nonviable core in single islet culture, and (2) loss of viability in high density culture. As islet loading density increases in a planar diffusion chamber, viable volume fraction decreases, and fully functional volume fraction drops dramatically.

[2] Maki T, Otsu I, O'Neil JJ, et al**.** Treatment of diabetes by xenogeneic islets without immunosuppression, use of a vascularized bioartificial pancreas. *Diabetes* 1996 45:342-7.

Insulin secretory capacity may increase little or even decrease as islet loading increases. Similar crowding effects can occur with encapsulated islets in the peritoneal space or with naked islets in the liver prior to vascularization if locally high concentrations of islets occur.

Single islets in culture have demonstrated several general characteristics:

- Oxygen uptake is associated with oxygen diffusion, and increases as islet size increases.
- When arranged in a single layer of islets in a square array, islets cultivated too close together develop non-viable regions.
- The density of cultured islets affects viability. As density increases, functionality declines quickly. Maximum performance occurs at relatively low density. Consequently, using a large number of islets may not be better than a smaller number.

## 2.3 Microcapsule Biocompatibility in Large and Small Animals

*David Hunkeler, PhD*
*Swiss Federal Institute of Technology*

A central issue in developing effective and biocompatible microcapsules is knowing the optimal size and membrane characteristics. As such, the biocompatability of various microbeads and membrane-containing microcapsules, all 400 μm in diameter, were examined when transplanted intraportally in rats.

A series of bead chemistries, based on alginates and various divalent salts, principally calcium and barium, were tested with explanations after three, seven, and 14 days revealing varying extents of cellular overgrowth, which were subsequently characterized. The best of these microbeads showed similar intrahepatic acceptance as microcapsules prepared using the alginate/cellulosesulfate/polymethylene-co-guanidine capsule chemistry, as well as alginate/polyvinylamine, indicating the acceptance of a polycation coating.

This is particularly relevant since the latter provides both immune and entrapment barriers and permits the tuning of the molar mass cutoff over the clinically relevant range of 10-250 kDa. The effect of depyrogenation and the endotoxin level of the anionic polysaccharides were also investigated. These experiments were used as the basis to monitor the acute and long-term (three month) effects of intraportal transplantation, at therapeutic dosages (10,000 capsules per kg of body weight) into large white pigs (25 kg). No long-term adverse effects were observed.

In conclusion, the optimal morphology of microencapsulated islets for given transplantation sites will continue to be an important area of investigation. However, current investigations do illustrate certain desirable material characteristics – that two polymers with rigid characteristics are not desirable, for example, since they do not work well together; and if using a combination of polymers, it is better if one is sensitive to pH.

## 2.4 Search for a More Perfect Immunobarrier System

*Taylor Wang, PhD*
*Vanderbilt University*

Immunoisolation is one of the great promises yet to live up to its full potential. Many capsules work well in rats and mice, but do not yet work in large animals, at least not consistently. In large animals, capsules tend to break or fibrosis develops around them.

This problem can be approached from a physicist's point of view, using tools common to the physical sciences. A case in point is the use of spherical shell technology involving multi-component polymer combinations in the search for a more prefect immunobarrier system.

In research on the dynamics of a compound drop in a low-gravity environment,[3] various mechanisms that are important to the encapsulation of islets have been discovered. For example, when a bubble is introduced into a droplet in the low gravity of space, over time the two can be driven into concentric geometry under a set of physical conditions. As a result of publishing the findings of these experiments, an interdisciplinary team of medical scientists, physicists, mechanical engineers, polymer scientists, and animal researchers were formed to find ways of making a more perfect immunobarrier system for medical uses. Their search led to a poly-methelene-co-guanidine (PMCG) system currently under study.

The team developed a technology in which the quality of a droplet can be controlled. By doing this, capsules can be produced that are virtually identical – very uniform, and with the same thickness. The precision control process allows further study of capsule formation and performances.

Recent large animal data on biocompatibility, mass transport, immunoprotection, and capsule stability have been very encouraging. However, Dr. Wang and his colleagues also found this problem to be very complicated, since 14 independent parameters are involved. Currently, a systematic appraisal process is being used, changing one parameter at a time to determine its effect on mechanical strength, permeability, and immunoisolation.

An effective, biocompatible capsule must have a strong membrane, and must be stable. It must also have good permeability and immunoprotection. Having a good system for manipulating capsule parameters will help find the optimal capsule parameters needed for successful transplantations of encapsulated islets. The research team is currently studying both dog and neonatal islets transplanted into dogs to explore these issues – about 100,000 capsules per 20-kg dog. From all the many possible combinations, a more perfect immunobarrier system for a large animal model may be found.

[3] Wang TG, Anilkumar AV, Lee CP, Lin KC. Core-centering of compound drops in capillary oscillations: observations on USML-1 experiments in space. *J. Colloid Interf Sci* 165:19-30; Wang TG, Anilkumar AV, Lee CP, Lin KC. Bifurcation of rotating liquid drops: results of USML-1 experiments in space. *J Fluid Mech* 276:389-403; Wang TG, Anilkumar AV, Lee CP. Oscillations of liquid drops: results from USML-1 experiments in space. *J Fluid Mech* 308:1-14.

## 2.5 Using PEG to Improve Biocompatibility and Permselectivity of Encapsulated Islets

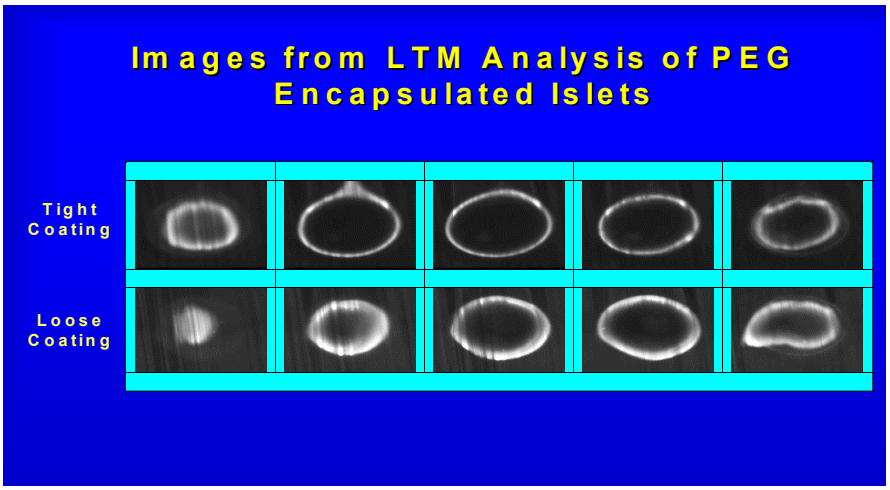
*David W. Scharp, MD*
*Novocell, Inc.*

While many encapsulated islet studies have been published regarding a variety of encapsulation methods, there has been little advancement of these islet encapsulation studies to effective results for the patient with insulin-requiring diabetes.

One of the major problems is that rodent results are not very predictive of large animal results. There may be a host of reasons for these differences, including different host responsiveness to the encapsulation materials, the immune response to the antigens releasing from the encapsulated islets, and site specific differences for islet survival. One of the inherent difficulties with many islet encapsulation technologies is the lack of ability to manipulate the basic encapsulation reagents and the final capsule or coating in terms of biocompatibility or permselectivity.

A successful islet encapsulation technology must demonstrate several vital characteristics including: biocompatibility; permselectivity (islet nutrition and function, prevention of rejection); relevant size and implant site; stability and safety; an ability for replenishment or replacement; and the source of the islets must be appropriate.

Polyethylene glycol (PEG) offers a range of possibilities in the selection of materials and coatings for islets. PEG comes in a wide range of monomer sizes from a few hundred kD's to millions of kD's. There also are a wide range of types of PEGs, from linear molecules to multiple-armed molecules. There is an inherent biocompatibility profile for each of these examples. These inherent biocompatibility profiles can also be markedly changed by incorporating many different types of molecules onto the monomer, onto one of the reagents, or incorporated into the polymer.




Thus, the application intended can dictate several choices of PEG molecules for use as well as a wide range of incorporating molecules to alter the biocompatibility of the final capsule or coating. In addition, depending upon the linking system used, one has additional choices for how to cross-link the monomer into the encapsulating polymer. This means that one can have a very wide control over the permselective profile of the encapsulating capsule or coating. These permselective profiles can be made quite narrow or very broad in terms of molecular weight proteins. PEGs offer other opportunities for manipulating permselectivity than simple varying the pore size in the cell coating.

Depending upon the PEG selected as well as adding reagents, one can control the hydrophobicity of the coating. One can also control the net charge of the coating from negative to neutral to positive. All of these choices provide wide options in biocompatibility as well as permselectivity for coatings or capsules designed for islets.

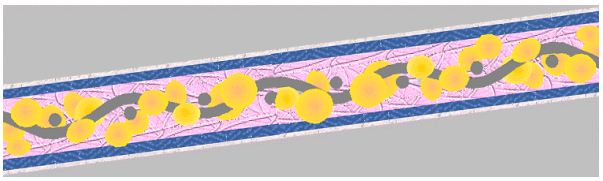
# 3. Industry Perspectives

Representatives of biomedical companies involved in islet encapsulation development discussed the status of experimental products during a session chaired by David Scharp, MD, of Novocell. The Islet Sheet being developed by Islet Sheet Medical, issues involving the use of alginates for encapsulation including transplant efficacy, and MicroIslet's encapsulation method using layers of highly purified alginate separated by a layer of a polyaminoacid were among the topics.

## 3.1 Host Response to the Islet Sheet, a Macroscopic Bioartificial Pancreas

*Rick Storrs, PhD*
*Islet Sheet Medical*

A treatment that produces euglycemia without risk of hypoglycemia or use of drugs would be widely regarded as an effective cure for diabetes. Among the more promising experimental approaches for achieving this are islets that are encapsulated in microspheres and islets within thin sheets.



**Islet Sheet cross-section**

Islet Sheet Medical (ISM), based in San Francisco, has developed an experimental planar, thin sheet bioartificial endocrine pancreas called the Islet Sheet, fabricated from highly purified alginate gel and viable islets of Langerhans. The design objectives for the Islet Sheet are to keep islets alive, prevent a destructive host response, and have a device that can assure practical surgical implantation. Among sites that appear to be suitable for the Islet Sheet are the liver, diaphragm and pancreas.

Acellular alginate layers form a uniform immunoprotective barrier to host rejection of the encapsulated cells, which are nourished by passive diffusion from adjacent host tissue. The overall thinness of the Islet Sheet, 250 μm, minimizes the barrier to nutrient diffusion.

Host foreign-body response limits the long-term utility of immunoprotective implant devices. Fibrotic reactions alter the stability and permeability characteristics of these implants, leading to device failure. Means to prevent deleterious host response have focused on polymer coatings to restrict direct cellular host contact with the foreign materials. However, the polymer coating itself can elicit immunological recognition, initiating a foreign body response culminating in formation of an impermeable collagen capsule.

ISM has utilized alginates as a versatile semipermeable-coating matrix for long-term implantation of cellular devices. In a pivotal study, a device measuring 4 cm by 8 cm containing a polyester scrim encapsulated in purified alginate and implanted in several intraperitoneal sites of a beagle dog remained substantially free of fibrosis for at least three months, providing sheet planarity was maintained.

In another experiment, approximately 75,000 allogeneic islet equivalents in six Islet Sheets were sutured to the omentum of a 7 kg female Beagle dog at the time of pancreatectomy. Fasting euglycemia was maintained for 84 days. Fed blood sugars were largely below 150mg/dL. A single injection of 2U insulin was administered on day nine, no other drugs were used. IVGTT data were not normal, but seemed to improve between 30 and 60 days.

Upon omentectomy and sheet removal, the metabolic parameters deteriorated to a frankly diabetic state within seven days. The sheets did not remain flat, but were recovered within hard, mostly acellular capsules. Dithizone staining islet clusters within alginate sheets were recovered from the interior of these capsules, suggesting that allogeneic islet tissue survived 84 days, protected by the Islet Sheet, and was responsible for maintaining fasting euglycemia. Similar studies using devices containing viable cells are ongoing.

Other current studies are examining the best implantation site, best method of attaching the sheets (suture or staples, glue, etc.), dose response, and longevity of function. Possible applications for Islet Sheets include primary allografts, expanded or engineered cells, possibly xenografts, and co-encapsulation with immunomodulators.

## 3.2 Alginate for Encapsulation: Functionality, Purity, Standards and Regulatory Considerations

*Michael Dornish, PhD*
*FMC BioPolymer (formerly Pronova Biomedical)*

Alginates are straight-chain polysaccharides derived from seaweed and kelp that have been used in a variety of products, ranging from simple technical applications such as viscosifiers to advanced biomedical matrices providing controlled drug delivery from immobilized living cells.

FMC BioPolymer, based in Oslo, Norway, has developed a sterile, ultrapure alginate that is easy to dissolve, easy to handle under sterile conditions, is ideal for microencapsulation of cells, and has low endotoxin content.

As for most hydrocolloids, the functionality of alginate is related to its chemical and structural composition. Since alginates are a heterogeneous group of polymers with a wide range of functional properties, the success of an immobilization or encapsulation procedure will rely on an appropriate choice of materials and methodology. This must be based on knowledge of the chemical composition of alginate and the correlation between structure and functional properties of the polymer. It is also important to recognize the need for working with highly purified and well-characterized alginates in order to obtain gels with reproducible properties.

The key characterization parameters of alginate can be grouped as follows:

- Innate characteristics describe the alginate molecule. Important parameters are mannuronate (M)/guluronate (G) composition, sequential structure, molecular weight and molecular weight distribution.

- Functional parameters, such as gelling, are a result of the M/G composition and the sequential structure of M and G along the alginate chain. In particular, the number of G's and the average length of the G blocks are important in determining the gel strength.

Sodium alginate is on the list of materials described as "generally recognized as safe" (GRAS) by the U.S. Food and Drug Administration (21CFR184.1724). This permits sodium alginate (but not other salts, such as magnesium) to be used in foods as a thickener or gelling agent, but does not indicate approval for the use of alginate in pharmaceutical and/or biomedical applications.

Therefore, regulatory parameters must also be addressed in medical applications that use alginates. For example, the American Society for Testing and Materials (ASTM) is making a concerted effort to establish standards and guidelines for the entire field of "tissue engineered medical products" (TEMPS). Safety, consistency and functionality of biomaterials used as matrices, scaffolds and immobilizing agents in TEMPS are a concern.

An ASTM alginate guide gives information on selection of testing methodologies and safety criteria. Critical parameters such as monomer content, molecular weight and viscosity, in addition to more general parameters such as dry matter content, heavy metal content, bioburden and endotoxin content are described in the ASTM document.

The presentation included a discussion of the innate characteristics of alginates, as well as the process of manufacturing, emphasizing how these factors will influence the performance of a final application.

In conclusion, criteria needed for successful use of alginate for islet encapsulation will involve product availability, good industry production standards, regulatory acceptance and approval, cost/benefits functionality, and adequate market development.

## 3.3 Factors Affecting the Transplant Efficacy of Islets Microencapsulated with Purified Alginate

*Will Bachalo, PhD*
*Islet Technology*

Individual encapsulation of islets with a coating that is selectively permeable (micro-encapsulation) appears to be the most promising approach for islet transplantation without requiring a life-long regimen of immunosuppressive drugs.

Coating materials must allow diffusion of nutrients, hormones, oxygen, and ions; allow glucose to reach the islets; and permit the insulin released by the beta cells to be readily passed out of the capsule as well as the metabolic waste. Whereas the islets have a highly developed rich network of capillaries within them when they are found in the pancreas, islet isolation results in the termination of this vascular system. Hence, the islets isolated for transplantation are dependent entirely upon diffusion for transfer of oxygen, nutrients, ions, hormones and metabolic waste to maintain the health of the islet.

At the same time, the coating material must provide an impermeable barrier to larger molecules and cells of the body's immune system. Because of the pore size of the coating material, the immunoglobulins and cytokines are prevented from diffusing through the membrane of properly coated islets. Although the immunoisolation membrane prevents passage of the immune cells and complement, antigens released by the transplanted cells can penetrate the membrane and activate the immune cells, resulting in the release of lymphokines such as IL-1 and cytotoxic agents such as free radicals, nitric oxide, and peroxides. The molecular weights of lymphokines are on the order of 20,000 daltons and hence, may be retained by the immunoisolation membrane. However, encapsulated islets must necessarily have a low tissue density in order to survive the limited diffusion flux of oxygen that is available. The lower concentration of encapsulated tissue results in a corresponding low concentration of shed antigens with a reduced concentration of soluble immune agents. Coating materials must also be nonfibrogenic so that adhesion of cells to the surface of the coating over time does not restrict the functioning of the pores.

Microencapsulation with purified alginate has evolved as a promising material for providing a noncytotoxic, non-biodegradable, selectively permeable barrier to immunological attack by the host. Encapsulation methods have been successfully developed for the islet tissue to maintain viability in the host, provide complete coverage of the islet, allow good insulin release dynamics, allow adequate oxygen diffusion to all cells in the capsule, and be permeable to small and medium-sized proteins while providing a barrier to the larger antibody molecules. Methods to be discussed have enabled the encapsulation of large numbers of islets (over 400,000 IEQs are required for human transplantation) with spherical diameters of from 200 μm to 600 μm.

Clinical transplantation of microencapsulated pancreatic islets is subject to several challenges. Small capsules must be produced with an adequate production rate and reliability. Methods developed have overcome these challenges with the current capability to consistently generate thousands of capsules per second at the required size. The requirement that there is a high probability of only one islet in each capsule inevitably leads to a significant percentage of blank capsules that need to be removed from the sample.

Development of diagnostics based upon laser-based electro-optical methods, including light scatter detection, will enable rapid online interrogation of each capsule to measure the capsule and enclosed islet size, the centering of the islet in the capsule, and the detection of any exposed tissue. Blank capsules and capsules not meeting the requirements will be sorted at a rate of from 100 to 1,000 per second. The processing will be performed while the encapsulated islets are in media under incubator conditions to minimize stress.

Islet Technology's clinical studies involving various mouse and dog models show promising results:

- Microencapsulated (Algi-Pure™ CapCells™) porcine islets Tx (March 2000) into peritoneal cavity of diabetic (Streptozotocin) C57 Bl/6 mice (eight mice confirmed diabetic at > 350 mg/dl). No immunosuppression was observed. Results: one mouse normal glycemic for 119 days, seven mice normal glycemic until end of life (348, 438, 481, 481, 488, 488, and 488 days).
- Microencapsulated (Algi-Pure™ CapCells™) dog islets Tx into peritoneal cavity of pancreatectomized diabetic dogs. No immunosuppression was observed. Results: March 1993 Dog – normal glycemic and insulin free for over seven years; and February 1999 Dog – normal glycemic and insulin free for 53 days.

Capsules retrieved from the mice were free from fibrotic overgrowth and the islets responded to glucose stimulation. Islet Technology, Inc. has had similar success with xenogeneic transplants of porcine islets in mice. These mice were made diabetic using streptozotocin and after transplantation with islets encapsulated with purified alginate, showed near-normal glycemia levels over their natural life-span.

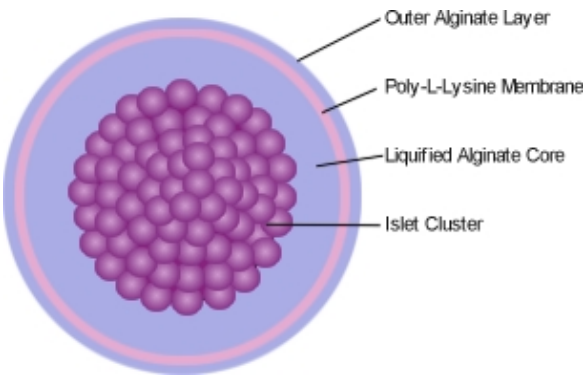
## 3.4 Microencapsulation of Islets for the Treatment of Type I Diabetes

*Ingrid Stuiver, PhD*
*MicroIslet, Inc.*

Immune destruction is one of the primary issues that must be overcome with respect to the transplantation of islets for the treatment of insulin dependent diabetes.

In order to circumvent the need for immunosuppressive drugs during islet transplantation, islets can be encapsulated using biopolymers that act as immunobarriers while permitting mass transfer of nutrients, glucose, and insulin to and from the islets.

MicroIslet, Inc. has utilized existing methodology, originally developed by Lim and Sun,[4] whereby islets are surrounded by two layers of highly purified alginate separated by a layer of a polyaminoacid such as poly-L-lysine. This technology utilizes chelating agents to liquefy the core, a process thought to improve the mass transfer of insulin and nutrients to and from the islet.




In the MicroIslet process, islets are first embeded into an alginate bead, providing a matrix for the islets; then a poly-L-lysine coating is added as a membrane to immunoisolate the islets. The outer alginate coating is added to make the capsule bio-compatible with the host. In addition to the process to liquefy the inner core to enhances mass transfer of nutrients, a salt treatment is also used to reduce capsule swelling and improves durability. This process treats the capsule with a salt solution. The MicroIslet capsules range in size from 400 μm to 600 μm.

Working with Duke University, MicroIslet has successfully transplanted capsules into a diabetic baboon, which returned to normal three days after implantation. The animal was normoglycemic and survived for 14 months. Studies are in progress to reproduce these data.

The goal at MicroIslet is to improve and streamline the encapsulation process further to generate a stable, biocompatible capsule that protects and promotes the longevity of the islets.

Key research issues include examining factors that cause capsule fragmentation *in vivo* and *in vitro*, finding ways to minimize inadequate encapsulation, developing process conditions to improve capsule strength, finding the optimum capsule size for optimum mass transfer, and understanding better the factors that maintain islet architecture. Of significant emphasis will be the development of isolation and encapsulation processes that maintain the islet in a near native state.

A successful capsule must be non-toxic and should be biodegradable. It needs to facilitate sustained islet viability and functionality within the capsule, while allowing optimum mass transfer of nutrients, hormones and growth factors. Such a capsule must be strong and show long-term durability; and it must not produce significant tissue reactivity. A reproducible encapsulation process, evaluation of bipolar half-life, and compliance with U.S. Food and Drug Administration guidelines are also important objectives.

[4] Lim.

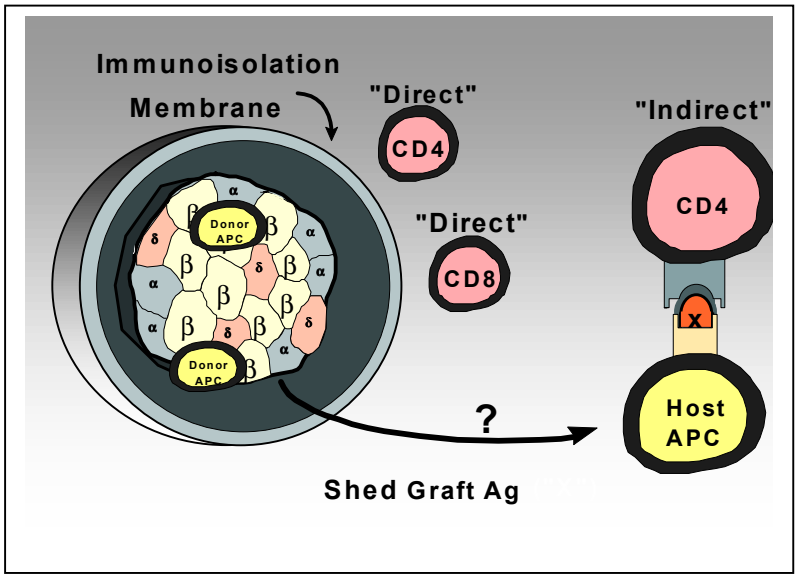
# 4. Immunoprotective and Anti-Inflammatory Therapies

Ray Rajotte, PhD, University of Alberta, chaired a session about immunoprotective and anti-inflammatory therapies. Cellular immunity to immunoisolated transplants, enhanced islet viability and co-encapsulation with Sertoli cells, and peptide blocking of apoptosis were examined.

## 4.1 Cellular Immunity to Immunoisolated Transplants

*Ron Gill, PhD*
*University of Colorado*
*Barbara Davis Center for Childhood Diabetes*

Immunoisolation has formed a long-standing approach to preventing the cellular or humoral rejection of islet allografts and xenografts. However, relatively little is known regarding the nature of immunity to encapsulated cells and tissues. A primary premise is that immunoisolation is not actually antigen isolation, per se, but rather is *cell* isolation. That is, while synthetic membrane barriers are designed to prevent cell-cell contact between host and donor cells, soluble antigens derived from the transplant are likely to gain access to the host immune system.




The design of immunoisolation technology requires considering the types of T cell responses that can occur in response to the graft. Given the T cell-dependent nature of graft rejection, one must consider two distinct forms of T cell graft recognition: “Direct” (or donor MHC-restricted) antigen presentation in which T cells engage native donor MHC-peptide complexes expressed on the surface of graft-derived antigen-presenting cells (APC); and “Indirect” (or host MHC-restricted) antigen presentation in which donor-derived antigens are captured by recipient APCs, degraded, and re-presented in association with recipient MHC molecules.

Importantly, exogenous antigens are generally processed and presented by class II MHC molecules and so invoke a predominantly CD4 T cell response. Thus, while tissue isolation strategies that prevent cell-cell contact between donor cells and host immune cells are expected to block the direct pathway, the indirect pathway is expected to be more problematic. That is, despite the encapsulation barrier, there is still the potential for graft peptide antigens to traverse the isolating membrane and be presented by host-type APCs.

A major unanswered question centers on the extent of such indirect CD4 T cell reactivity to immunoisolated allografts and xenografts. Generally, immunoisolation has been more successful for allografts than for xenografts. Such results are consistent with many observations that islet allograft

rejection requires 'direct' recognition while exaggerated 'indirect' T cell reactivity occurs in response to xenografts. The degree and consequence of the 'indirect' T cell response to immunoisolated tissues is largely undefined, but this pathway would account for studies showing that CD4 T cells can participate in the rejection of encapsulated islet xenografts.

Thus, while preventing cell-cell contact through encapsulation technology is currently realistic, preventing potential 'indirect' presentation of small molecular weight donor-derived antigens may not be readily feasible. At present, this would imply that encapsulation would preferentially facilitate allograft rather than xenograft survival.

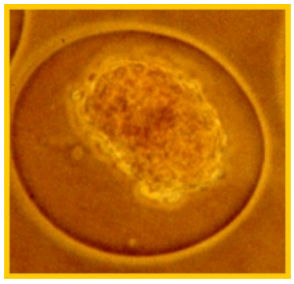
Additional attempts to reduce capsule porosity substantially in order to restrict the molecular mass of molecules traversing the size-selective barrier may not be warranted without clear evidence that defined cellular and humoral pathways or candidate effector molecules are indeed attenuated by this strategy. Otherwise, an unacceptable sacrifice in graft viability/nutrient exchange may be made without a corresponding benefit in immune protection. Rather, adjunct immunomodulatory therapies may be required to optimize the survival of encapsulated tissues, especially in the case of xenotransplantation.

## 4.2 Alginate Microencapsulation: Enhanced Islet Viability and Co-encapsulation with Sertoli Cells

*Ray V. Rajotte, PhD; Greg Korbutt, PhD; Jannette Dufour, PhD*[5]
*University of Alberta*

Using the Edmonton Protocol, clinical islet transplantation can now produce insulin independence in 100 percent of brittle diabetic patients. To make this the treatment of choice for all type 1 diabetic patients, two major problems need to be resolved:

- develop an unlimited source of islet tissue
- transplant without continuous immunosuppression



Encapsulated islet.

Over the last 30 years, many approaches have been proposed to address the immunosuppression problem, including islet encapsulation. Considerable effort and funds have been devoted to islet encapsulation. This technology, however, still remains clinically inapplicable.

There have been isolated cases of success in small and large animals, but these studies have been difficult to replicate. This variability is in part due to the methods of encapsulation as well as differences in the biomaterials used – which often provoke an inflammatory response.

In an effort to address why encapsulated islet grafts fail, a University of Alberta study was conducted to differentiate between immune failure versus biological failure. Encapsulated islets were transplanted into diabetic immunoincompetent nude mice. Surprisingly, we found that a highly purified alginate open pore capsule (Metabolex, Inc.) enhanced islet viability, and fewer islets were needed to correct the plasma glucose post-transplant. We also found this alginate matrix enhanced islet viability *in vitro*. This observation has also been seen in research by others, suggesting islet failure is immunological.

[5] Dr. Rajotte presenting.

To develop a perfect immunoisolation device that will protect islets from allograft and xenograft rejection as well as autoimmune destruction will be difficult. One strategy utilizes Sertoli cells, which secrete immunosuppressive factors and are able to protect themselves as well as islets from rejection.

We co-encapsulated islets and Sertoli cells with an open pore bio-compatible alginate matrix and found the Sertoli cells protected the allografted rat islets long term. This, however, now needs to be tested in a large animal model. Co-encapsulation may be a way of creating a microenvironment that protects the islets from immune attack while improving islet viability.

## 4.3 Peptide Blocking of Apoptosis to Preserve Beta Cell Function

*Christophe Bonny, PhD*
*University of Lausanne*

Recent research at the University of Lausanne has demonstrated that the c-Jun N-terminal kinase JNK, which is activated by proinflammatory cytokines (IL-1β, TNFα), is required for the death of pancreatic beta cell lines exposed to IL-1β.

Cell-permeable peptide inhibitors of JNK, which are able to protect pancreatic beta cell lines against IL-1β induced cell-death, have recently been produced. A protease resistant D-enantiomeric form was engineered (D-JNKI) and has been shown to remain inside cells for up to two weeks, where it continuously protected cells against exposure to IL-1β.

These inhibitors efficiently penetrate isolated rat pancreatic islets, where they inhibit JNK signaling. The peptides significantly reduce IL-1β-induced rat islet death. Furthermore, surgical stresses have been demonstrated to be extremely potent activators of JNK. When added at the earliest stage of the rat islet isolation procedure, the D-JNKI peptide significantly increases the number of islets recovered after culture.

These data indicate that the prolonged blockage of JNK through the use of cell-permeable peptides may both increase islet yield and confer protection against cytokine-induced destruction. The system might also be used for the efficient and sustained delivery (days to weeks) of other protective chemicals.

# 5. Gene Therapy for Islet Immunoprotection

A session on immunoprotection through gene therapy was led by Bernard Thorens, PhD, University of Lausanne. Engineering transplantable Beta cells using lentiviral transfer, adeno- or lentiviral vectors to transfer protective genes into islets, and immunomodulation of Beta cells in transgenic mice using adenovirus E3 genes were discussed.

## 5.1 Systematic Engineering of Transplantable Beta Cell Lines Using Lentiviral Vectors

*Bernard Thorens, PhD*
*University of Lausanne*

Function and survival of insulin secreting cells encapsulated and transplanted in a type 1 diabetic environment are limited by metabolic and inflammatory stresses. A fundamental issue is whether it is possible to engineer genetically to improve the survival of these encapsulated cells – better resistence to hypoxia, for example, and better resistence to inflammatory stimulation.

Research at the University of Lausanne is exploring the possibility of improving, systematically, by genetic engineering, the resistance of these insulin secreting cells against the transplantation conditions. As a model system, the ßTc-Tet cells, which can correct diabetes in syngeneic or allogeneic mice when transplanted under the kidney capsule, are being used.

The research has demonstrated that transferring the Bcl-2 anti-apoptotic gene using lentiviral vectors did not interfere with glucose-stimulated insulin secretion, but conferred increased resistance to hypoxia, growth at high cell density, and partial resistance to cytokines killing. Furthermore, expression of Bcl-2 was essential for cell survival in flat sheet encapsulation devices.

These modified cells (referred to as CDM3D) were then used to transfer additional genes conferring resistance to specific cytokines. The general approach was to overexpress genes known to interfere with the intracellular signalling pathways activated by IL-1ß, IFN-γ and TNF-α. This was achieved by transducing, respectively, dominant negative forms of the adaptor protein Myd88 (Myd88-Toll and Myd88-lpr), SOCS-1, and v- and c-FLIP. *In vitro*, it was demonstrated that this systematic approach was successful in blocking the decrease in glucose stimulated insulin secretion induced by the cytokines and in protecting the cells against apoptosis induced by the respective cytokines.

These cells are now being tested in transplantation experiments in allogeneic and NOD mice to determine the protective effect of each genetic modifications. Cells are transplanted unencapsulated or encapsulated in flat sheet devices and, for comparison, in alginate microbeads.

## 5.2 Adeno- or Lentiviral Vectors to Transfer Protective Genes into Isolated Islets

*Massimo Trucco, MD*
*Children's Hospital of Pittsburgh*

The autoimmune nature of insulin-dependent (type 1) diabetes targets the beta cells of the pancreas for destruction. Because of the absence of a sufficient number of beta cells, the patient requires a lifelong commitment to insulin replacement.

Different approaches have been attempted to eliminate the requirement of exogenous insulin administration, including pancreas and islet transplantation. Allogeneic islet transplantation, which is the more suitable treatment of the two surgical procedures in younger diabetics, faces a hostile immunological response, consisting of recurrent autoimmunity and allogeneic rejection. Pharmacological immunosuppression, even that implemented in the Edmonton protocol, is not optimal for preserving the transplanted islets in children since drug toxicity is predicted to emerge with time, not only for the transplanted tissues but also for the recipient's kidney and liver.

Gene therapy-based approaches add a new dimension to the efforts aimed at blocking this compounded immunologic attack without the need for pharmacologic immunosuppression. A possible approach consists of transducing *ex vivo* the islets isolated from a donor with immuoregulatory genes before transplanting them into the diabetic recipient. The most promising genes, alone or in combination, and vectors should be tested to determine the most effective ones before attempting to translate the therapeutic protocol from rodents to humans.

With regard to the most promising genes, researchers at Children's Hospital of Pittsburgh tested the effect of anti-apoptotic (e.g., inhibitor of κB [IκB], and insulin-like growth factor 1 [IGF1]), oxygen radical scavenger (e.g., Manganese Superoxide Dismutase [MnSOD]), and soluble anti-inflammatory (e.g., Interleukin 1 Receptor Antagonist Protein [IRAP], and Indolamine,2-3-dioxygenase [IDO]) genes.

To obtain proof of principle, adenoviral vectors were used first. The same genes were then transferred into a lentiviral vector, whose benign antigenicity and stable-integration character are superior to the used adenovirus. Although each gene encoded by the adenoviral vector showed the expected positive effects, both *in vitro* and *in vivo*, none of the ones tested were sufficient per se to preserve the islets indefinitely from a prediabetic NOD mouse once transplanted into an NOD*scid* mouse that was subsequently given splenocytes from a diabetic mouse.

Combinations of more than one gene showed an improved effect even in this test, certainly immunologically more demanding than the characteristic pathologic process. Here again, however, the treatment did not confer to the transplanted islets a protection that survived the challenge of time.

The use of more appropriate vectors in terms of persistence of the transfected genes, reduced antigenicity, and safety for the recipients, which are currently being tested, include the Adeno-Associated virus (AAV) and the Equine Infectious Anemia virus (EIAV), which is not infectious in humans.

Perhaps the final solution of the problem can be found in a protocol that uses gene therapy in combination with additional immunoregulatory means.

## 5.3 Immunomodulation of Beta Cells in Transgenic Mice Using Adenovirus E3 Genes

*Shimon Efrat, PhD*
*Department of Human Genetics and Molecular Medicine*
*Sackler School of Medicine, Tel Aviv University*

Viruses constitute a rich source of immunomodulatory genes, which allow infected cells to escape immune surveillance. These genes could be used to increase the resistance of transplanted cells to immune rejection, as well as in gene therapy of cells *in vivo* aimed at reducing their susceptibility to autoimmunity.

The early 3 (E3) region of adenoviruses encodes a number of immunomodulatory proteins, which interfere with class I major histocompatibility-mediated antigen presentation and confer resistance to cytokine-induced apoptosis in cells infected by the virus. Expression of the E3 genes in beta cells in transgenic mice under the insulin promoter (RIP-E3) allowed transplantation of islets from mice into allogeneic strains and provided protection from autoimmune destruction in a cross with RIP-LCMV mice, a model of virus-induced autoimmune diabetes.

Recently, transgenic expression of the RIP-E3 transgene in NOD mice was shown to decrease the incidence and delay the onset of autoimmune diabetes.

Taken together, these findings demonstrate the potential of E3 genes for beta-cell immunomodulation. E3 genes introduced into isolated islets or beta-cell lines may increase their resistance to graft rejection. In addition, expression of E3 genes in beta cells in prediabetic islets may prove an efficient approach for preventing type 1 diabetes.

# 6. Recommendations

A select group that included session chairs and sponsors developed major recommendations emerging from the presentations and discussions. The group generally agreed that there are two high-priority issues: the need for successful large animal studies for further evidence and ultimate validation, and standardization of capsule materials and implantation procedures. Within that framework, statements identifying specific research objectives were developed.

## 6.1 The Need to Demonstrate Success in Large Animal Models

*David C. Klonoff, MD, FACP*
*Mills-Peninsula Health Services, San Mateo, CA*
*University of California at San Francisco*
*Editor-in-Chief,* Diabetes Technology & Therapeutics

The need to demonstrate success in a large animal model is a major goal for microencapsulated islet research. The current status of the bioartificial pancreas is that the results are good in rodents, limited in large animals, and absent in humans. The immune systems of rodents, including mice and rats, are more tolerant to the implantation of tissue from themselves, from the same species, and from another species.

These small animals are also more immune tolerant to implantation of an inert membrane that would be used to encapsulate these cells than are the immune systems of larger animals. Demonstration of success of implantation of a bioartificial pancreas in a large animal (such as a dog, monkey, or ape) should be the goal of future research in development of the bioartificial pancreas. In order to receive research funding, an investigator should demonstrate the capability to implant encapsulated islet cells successfully into a rodent, but the emphasis on future funding is to improve and develop a system for implantation into a large animal. A successful large animal model would then be the basis for trials of encapsulated islet cells in humans.

Furthermore, in order to create a successful bioartificial pancreas, more data are needed as to why current systems for implantation of these devices have been poorly tolerated in large animals. As elaborated upon under other objectives that follow, areas of potentially fertile investigation include:

- a determination of an optimal chemical structure for the capsule
- quantification of the degree of purity of he biomaterials used (e.g. endotoxin proteins, metals)
- the optimal shape and size of the capsule
- the amount of oxygen and other nutrients necessary to nourish the encapsulated cells
- the nature of the immune signaling process whereby encapsulated islet cells release small molecules that attract host lymphocytes
- the use of engineered cell lines as a substitute for native islet cells
- the determination of an optimal implantation site, and method for removing old implants to avoid sensitization to the capsule material, and to prevent portal hypertension if they are released into the portal vein circulation.

## 6.2 The Cell Biology of Encapsulated Islets

*Gordon Weir, MD*
*Joslin Diabetes Center*

Much remains to be learned about what happens to avascular and denervated islets that are transplanted in capsules. Normal islets in the pancreas vary in size from being very small, consisting of only a few cells, to being very large, with some being over 400 μm in diameter. The average islet diameter in most mammalian species is about 125 μm, with the beta cells being of variable age, functional capacity and vulnerability.

Once islets are encapsulated, they are dependent for their oxygen supply and nutrition upon the microvasculature adjacent to the outer surface of the surrounding capsule. There are many questions about what happens to encapsulated islets even in the absence of immune attack, as can be studied experimentally using syngeneic transplantation models. Based on assumptions about oxygen diffusion and utilization by the outer cellular layers of the islet, we know that large islets (those over 200 μm in diameter) will have necrotic centers secondary to hypoxia. It is also important to realize that even without cell death, insulin secretion is markedly impaired in the presence of hypoxia. Therefore, islet cells may survive and look healthy but not secrete insulin efficiently in response to a variety of secretagogues, including glucose. Another issue concerns the intraislet relationship between beta cells and non-beta cells, these latter cells being found in the outer mantle cells of islets. It is thought that blood and interstitial fluid normally flow from the center of the islet to the mantle so that glucagon-containing cells are bathed in insulin, but beta cells see little, if any, glucagon from the mantle. This is probably fortuitous because glucagon is such a strong beta cells secretagogue. With encapsulated islets, glucagon secreted by alpha cells can presumably reach the beta cells in the islets, which may change their secretory function and even change their responsiveness to glucose.

Another major question concerns the maintenance of beta cell mass in capsules. How is it possible for encapsulated beta cells in mice to last for over 350 days? There is probably some low rate of beta cell replication going on which is crucial and there may also be some stem cells that make new beta cells. Some find that encapsulated rat islets transplanted into syngeneic rats fail in two to three months, thus doing less well than the mouse islets in mouse recipients. Rats, therefore, may be a good model to use to understand why failure can occur and may provide clues about the disappointing results seen so far in monkeys. Thus, studies might be carried out to learn more about what happens to rat (and mouse) islets at all stages of the experiments, to characterize them after isolation with collagenase, after encapsulation, just before transplantation, on the day after transplantation, and then at later time points. These studies will be complex and involve determination of islet size, whether there is central necrosis, what happens to the beta cell to non-beta cell ratio, what happens to the beta cell replication rate, apoptosis and necrosis.

## 6.3 Function of Encapsulated Islets

*Gerold Grodsky, PhD*
*University of California at San Francisco*
*Advisory Editor,* Diabetes Technology & Therapeutics

Standardized *in vitro* methods are required to establish which characteristics of encapsulated islet-cell function are predictive of optimal successful transplant and which deteriorate with transplant failure. To date, this usually has involved testing of insulin secretion in response to glucose. Perifusion (or clamp) studies may be required to permit evaluation of biphasic insulin secretion, characteristic of normal islets. It is necessary to establish if rapid insulin release, important for normal glucose homeostasis, is required

and how it is affected by islet-cell integrity and diffusion rates of insulin out of the capsule. More data is needed to determine insulin diffusion rates within various types and sizes of capsule. Response to other secretagogues should also be standardized and tested for predictive value for successful transplant. These may include c-AMP enhancing agents, depolarizing agents, amino acids, and fatty acids – alone or in combination. Insulin synthetic rates (pulse-chase) are rarely measured and could prove to be of predictive value.

More data is needed to establish the metabolic pathways required for successful transplant. Although it is clear oxygen availability is critical, more information on the significance of the various glucose metabolic pathways and oxidative-phosphorylation for successful transplant and continued function need testing. Although fatty acids serve as major fuels for islets, the significance of fatty acid metabolism for encapsulated islet-cell function has not yet been evaluated. The importance of a limited availability, *in vivo*, of albumin-bound fatty acids and chylomicrons needs testing. Knowledge of limiting or vulnerable metabolic steps could prove to be of value in designing engineered cell lines to enhance these sites (e.g., glucokinase; enzymes to increase respiration and reduce anoxia).

Finally, additional studies are required to determine which sites for islet transplant are optimal for fuel and secretagogue availability, and insulin and waste product release. Establishment, *in vitro*, of the role of flow rates around encapsulated islets on these parameters may be useful for optimal site prediction.

## 6.4 Characterization of Polymeric Biomaterials in Use for Immobilization

*David Hunkler, PhD*
*Swiss Federal Institute of Technology*
*Laboratory of Polyelectrolytes and Bio-Macromolecules*

Immunoisolation involves the selection of one or more polymers that must be compatible with the tissue to be encapsulated, while not provoking an immune reaction.

There are three main directions for the characterization and standardization of biomaterials:

- the *pure chemistry* (chemical structure) and molecular parameters (molar mass, charge density, molecular architecture, etc.)
- the *characteristics of the complexes* formed from polymers and/or multivalent ions/polyions (mechanical stability, permeability, biocompatibility)
- *purity* of the complex components and the complexes (e.g. concentrations and thresholds for endotoxin, proteins and heavy metals)

It has to be considered that the biocompatibility of the pure polymers can change if they are complexed. Moreover, the same polymer can behave different with various partners. Therefore, the need to investigate the macromolecule, and various binary complexes exists as each pair could be, reasonably, treated as a separate biologic from a regulatory perspective.

With the exception of alginate, for which purification and characterization have advanced (though are incomplete), little is known for other potential biomaterials, polysaccharides or synthetics alike. For example, while endotoxin reduction and characterization, as well as an assessment of the protein levels in alginates have been carried out, there is no ability to correlate these levels with the suitability of a material for transplantation, let alone its efficacy in islet transplantation. Furthermore, most alginates that are commercially available do not have heavy metal contents assessed, and have varying levels of mannuronic and guluronic acid residues, as well as different viscosities.

The situation is much worse for other potential polymeric biomaterials, which remain uncharacterized with regard to the aforementioned attributes, and have higher endotoxin levels, even following purification. Overall, one could conclude that biomaterial quality control is poor (between different batches, suppliers, or raw material sources such as sea weeds) and advances in characterization will likely be required.

Key research issues for alginates, cellulose sulfates, and other natural and synthetic water soluble polymers considered for encapsulation include the following:

- What is the level of endotoxin required, *cetibus paribus*, to reduce inflammation to an acceptable level for empty capsules transplanted into various sites (peritoneum, sub-Q, liver, omentum)?
- What are the key contaminants to polymer solutions, other than endotoxins, that provoke immune reactions?
- Can these key contaminants be reduced without a change in the physical properties of the polymer solution (to avoid introducing physical properties detrimental to encapsulation)?
- How should purified and depyrogenated polymers, or their solutions, be handed, both during storage and transplantation?
- Are there some polymers, or microstructures, for which the depyrogenation and purification will be more facile?

Research should be sponsored that seeks to characterize polymeric biomaterials contaminants that provoke inflammation upon implantation or transplantation, as well as the most suitable means to remove such contaminants. This can be imagined on key polysaccharides that have undergone clinical trials, including alginate and cellulose sulfate, as well as other materials that have shown preliminary biocompatability, such as xanthan, poly-L-lysine, polymethylene-co-guanidine and polyvinylamine.

Such experiments could be verified in small animal models, with the aim of progressing eventually to larger animal studies. It is clear that the bioartificial organ field, in general, lacks access to any batches of transplant-proven polymers. This is the major weakness from the material perspective and one that should be addressed in the long term.

## 6.5 Preservation of Beta Cell Function in Capsules, including Bioengineering of Cell Lines

*Bernard Thorens, PhD*
*University of Lausanne*
*Institute of Pharmacology and Toxicology*

Reduction in islet viability occurs at several steps during the encapsulation/transplantation procedure, from islet isolation, to *in vitro* culture, cell encapsulation and transplantation in a diabetic milieu. Beta cell mass is lost due to a multitude of events: anoikis, i.e., suppression of the interaction of the islets with their extracellular matrix during pancreas digestion; stresses due to changes in metabolic environment and probably loss of exposure to growth factors present in the pancreas. Furthermore, encapsulation places the islets in an unnatural environment where access to oxygen and nutrients is limited and absence of a normal extracellular matrix may impair structure and function. Finally, transplantation of the encapsulated cells in a type 1 diabetic milieu may reactivate the autoimmune system against shed antigens and induce local inflammatory reactions characterized, among others, by the release of cytokines and reactive oxygen species that limit encapsulated cell function and survival.

Intervention should be proposed to preserve survival and function of the beta cells during the isolation procedure*, in vitro* culture and following transplantation in diabetic patients.

The pathways inducing apoptosis and cell death in beta cells during the different steps of isolation, cell culture, and transplantation need to be better defined. Innovative methods to interfere either transiently or permanently with these death-inducing pathways should be proposed. These may include viral and non-viral technologies to transfer protective genes or use small peptide or non-peptide molecules to interfere with these pathways. These techniques should be amenable to large-scale treatment of isolated islets for transplantation in large animals or human. These techniques could also be designed for improvement of appropriate insulin secreting cell lines.

## 6.6 Characterization of the Immune Response to Encapsulated Islets

*Ronald G. Gill, PhD*
*University of Colorado*
*Barbara Davis Center for Childhood Diabetes*

Varied biomaterials have been used to provide an immune barrier for the transplantation of cells and tissues. Although conceptually important, much research in this area has been empirically driven in that the primary endpoint has been the duration of cell or tissue survival and function. While there have been dramatic successes reported in which immunoisolated allogeneic or xenogeneic tissues can survive in the absence of host immunesuppression, the reproducibility of such results appears to be limited. Also, a significant gap in knowledge in this field is the relative lack of information regarding the nature of host immunity that may contribute to the dysfunction of immunoisolated transplants. The design and improvement of immunoisolation strategies will greatly benefit from clarification of the host responses that are rate-limiting for the success of immunoisolation.

Major research objectives in this area include the following:

- Determine the contribution of innate immunity to the dysfunction of encapsulated cells and tissues. Also needed is a determination of key molecular components in biomaterials and/or transplanted tissues that trigger inflammatory responses from myeloid lineage cells.

- Determine the nature and contribution of antigen-specific (adaptive) immunity to the injury of encapsulated tissue allografts and xenografts. Such studies would include addressing the role of T and B lymphocyte responses, the nature of host antigen-presenting cells in triggering host lymphocyte responses, and the degree and consequence to graft survival of cellular versus humoral antigen-specific immunity to encapsulated tissues *in vivo*.

- Determine the properties of donor allogeneic or xenogeneic antigens capable of triggering host immunity to encapsulated tissues. For example, the molecular mass and characterization of potentially key donor-derived antigens that may egress from the encapsulated tissues would be considered significant.

- Determine key immune effector mechanisms that contribute to the demise of encapsulated allografts and xenografts. Identification of soluble effector molecules such as cytokines, inflammatory mediators, antibodies, and complement components that contribute to the injury of encapsulated transplants *in vivo*.

- Examine the application of adjunct induction therapies aimed at modifying host immunity and/or inflammation that facilitate the implantation and long-term survival of encapsulated allografts or xenografts.

- Develop gene-transfer approaches for rendering encapsulated cells or tissues resistant to injurious host responses.

## 6.7 The Role of Encapsulation Permeability Control

*David Scharp, MD*
*Novocell*

There may be significant differences in the importance of encapsulation permeability between encapsulated islet allografts and xenografts. It has been suggested that for allograft survival, it is only required to separate the donor cells from the recipient immune cells. There is evidence in the literature in rodent implant studies and one primate implant study that this may be sufficient for Type II diabetes models. There is also evidence in the Type I model including autoimmunity in the NOD mouse that simple cell separation may be sufficient. Whether this holds for human autoimmune diabetes is unknown.

Since it is anticipated that islet implants will have to be replaced on a regular basis due to limitations of growth of the islet cells, one has to consider what may happen with the subsequent encapsulated implants when considering the role of coating permselectivity. If the host is sensitized as a result of the first islet implant, which is likely, then with the second implant, the antigens crossing the immune cell immune barrier may still be responded to by the host antibodies and beta cell response. This response outside the capsule may be sufficient to mount additional host immune cell responses that cause fibrosis around the encapsulated cells that lead to their destruction by cytokine and hypoxic conditions.

There is no direct evidence available to anticipate the human responses to all three conditions in the Type I diabetic recipient: allograft rejection, autoimmune destruction, and sensitized allograft recipient. Only one clinical study has been performed with encapsulated islets using a coating permselectivity of 60 kD that demonstrated no difference between Type I and Type II diabetics and non-diabetic recipients responsiveness to encapsulated islet allografts. When one considers the encapsulated islet xenograft, additional questions arise as to the response to these cells and whether control of capsule permselectivity may be of additional help in reducing the host response. In both the sensitized islet allograft and the islet xenograft, the ability to reduce the size of antigens may be helpful if there is a family of antigens, such as the HLA antigens, that are specifically focused to the host immune system. If this is the case, the reduction of encapsulation permselectivity to below these antigen sizes, ~60 kD, may reduce the host response in these circumstances.

Unanswered questions include the following:

- Does the autoimmune Type I diabetes in humans require more than prevention of contact between donor cells and recipient immune cells to permit successful islet allografts?
- Is there a difference between human Type I and Type II diabetes responses to encapsulated islet allografts and can control of capsule permselectivity reduce any of these responses?
- If the human encapsulated islet allograft causes sensitization to the diabetic host with the first implant, is that host response reduced by reducing the cell encapsulation permselectivity for subsequent implants?
- Does control of cell encapsulation permselectivity play a significant role in encapsulated islet xenografts?

A primary research priority should be directed at establishing reliable models of Type I and Type II diabetes that can readily begin to answer these questions and begin to study the mechanisms of these responses. A great deal of information can be accomplished in the proper rodent models using different coating permselectivity settings between primary islet allografts, sensitized islet allografts, and islet xenografts.

This still leaves unanswered the question of the human models for encapsulated islet implants. There should be another priority to establish the appropriate humanized mouse model to be able to study these same reactions of the human immune response by reconstitution of the immune incompetent mice with human immune cells from patients with Type I and Type II diabetes as well as human immune cells from patients without diabetes. These reconstituted, humanized mice models may then be able to answer these questions prior to human clinical trials. The most important of these questions can then be answered in the diabetic primate model to determine whether the rodent results are predictive of the intact human immune response. Unfortunately, there is no autoimmune diabetes model established in the primate, so the questions will need to be limited to the other topics.

## 6.8 Co-encapsulation of Islets

*Ray V. Rajotte, PhD*
*University of Alberta*
*Surgical-Medical Research Institute*

Islet encapsulation technology has been around for 20 years and yet this technology has not been realized clinically. Much of the work that has gone on in the past has been difficult to reproduce because of non-standardized materials and lack of information on the processes used to encapsulate the islets (in part scientists wanting to protect potential intellectual property or companies not wanting to share trade secrets). When encapsulated islets are transplanted, two factors come into play – both of which affect islet viability. On one side, there is islet survival as it relates to nutritional factors that may lead to cell death; while on the other side, there is the immune responses that come into play.

To make a capsule that will protect the islets from all immune and inflammatory factors may be very difficult. One strategy that may solve some of the above problems is to co-encapsulate islets with cells that secrete immunosuppressive or anti-inflammatory products. These cells could be engineered or could be a body cell that secretes these products. Sertoli cells in the testes are nurse cells that secrete growth factors, immunosuppressive products, etc., and survive in low PH and in low oxygen, and are able to protect themselves from immune attack when transplanted as a xenograft. Co-encapsulation of islet and Sertoli cells might be one way to create a microenvironment that will protect the islets from the various immune and inflammatory factors that come into play when the encapsulated islets are transplanted.

Major research areas that need to be addressed include:

- encapsulated islet failure – nutritional, immunological, or inflammatory
- improved biocompatibility of the materials
- identify factors that lead to capsule failure
- identify cells that could be co-encapsulated with islets that may enhance survival
- identify transplantation site that gives long-term survival, which maintains physical control of glucose.

## 6.9 Oxygen Needs for Encapsulated Islets

*Gordon Weir, MD*
*Joslin Diabetes Center*

The oxygen needs of cultured and encapsulated islets is currently better understood because of the work from Clark Colton, PhD, and his colleagues at Massachusetts Institute of Technology. Islets in the pancreas are normally richly vascularized, but when isolated are completely dependent upon oxygen diffusion from the surrounding media or buffer. Moreover, when encapsulated and transplanted, they are dependent upon whatever oxygen can be delivered from adjacent capillaries in the transplant site. It is not only essential to consider the loss of cells from hypoxia, but reduced performance in terms of insulin production from surviving cells receiving only a marginal supply of oxygen.

To appreciate the complexity of oxygen needs in tissue culture, one must consider not just single islets but also the competition for oxygen by groups of islets. For example, a single islet of 200 μm in diameter in tissue culture will have some necrosis of the central core of beta cells. The same size or even smaller islet surrounded by other islets would be even more oxygen-starved. The health of islets in culture before encapsulation and transplantation should be taken into account, because it seems likely (although not yet proven) that islets under the stress of hypoxia in culture will do less well when transplanted. The fate of islets in tissue culture will depend upon the density of islets as a function of surface area of media and the depth of media, but it must also be recognized that islets do not remain equidistant from each other, so any tendency to clump will lead to more dysfunction and cell death.

Similar oxygen supply issues are found with encapsulated islets. For example, with islets in alginate microspheres placed in the peritoneal cavity, islets should do best if the capsules do not clump or have inflammatory cells on their surface. With surrounding inflammatory cells, islet cells might be damaged by production of toxic cytokines but suffocation of islets by oxygen consumption of these surrounding cells may be an even greater threat. Similar, but in some ways different concerns, face the approach of macroencapsulation, whether with planar sheet devices, alginate slabs, or hollow tubes. Work has already begun on modeling these more complex configurations to learn more about the predicted oxygen availability for islets in such devices. For example, the modeling of a slab of cells 50 μm thick inside a planar device is very complex in terms of predicting cell survival and function, but predictions can be made and tested. Another variable will be the site of implantation, as there must be differences between peritoneal, subcutaneous, hepatic, and other locations. Because of these complexities and limitations, it will be valuable to make theoretical estimates of what level of islet function can be supported inside devices and then to perform experiments to test these assumptions.

# Appendix A: Workshop Agenda

**December 6-7, 2001 – Washington, DC**

sponsored by

Juvenile Diabetes Research Foundation International (JDRF)
National Aeronautics and Space Administration (NASA)
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
National Center for Research Resources (NCRR)

| | **Day 1 — Thursday, December 6** | |
|---|---|---|
| 7:30 – 8:30 | Continental Breakfast | |
| | **Session I: Introduction of Key Issues** | |
| | Session Chair: David Klonoff, MD, *University of California – San Francisco* | |
| 8:30 – 8:40 | Welcome and Introductory Remarks | Charles Queenan III<br>*Chair of Research, JDRF* |
| 8:40 – 8:55 | The Promise and Challenges of the Bioartificial Pancreas | David Klonoff, MD<br>*University of California – San Francisco* |
| 8:55 – 9:20 | Overview of Challenges to Advancing the Bioartificial Pancreas to the Clinic | Miriam Provost, PhD<br>Steven Bauer, PhD<br>*U. S. Food & Drug Administration* |
| | **Session II: Encapsulation and Immunobarriers** | |
| | Session Chair: Gordon C. Weir, MD, *Joslin Diabetes Center* | |
| 9:20 – 9:35 | Protection from Immune Destruction | Gordon C. Weir, MD<br>*Joslin Diabetes Center* |
| 9:35 – 9:50 | Oxygen Requirements of Islets | Clark Colton, PhD<br>*Massachusetts Institute of Technology* |
| 9:50 – 10:05 | Microcapsule Chemistry and Transplantation Site Selection/Correlation: Rodent and Pig Experiences and Experiments | David Hunkeler, PhD<br>*Swiss Federal Institute of Technology* |
| 10:05 – 10:35 | *Break* | |
| 10:50 – 11:05 | Search for a More Perfect Immunobarrier System | Taylor Wang, PhD<br>*Vanderbilt University* |
| 11:05 – 11:20 | Using PEG to Improve Biocompatibility and Permselectivity of Encapsulated Islets | David Scharp, MD<br>*Novocell* |
| 11:20 – 12:00 | Questions, Answers, and Audience Discussion | |

| | | |
|---|---|---|
| | **Session III: Lunch Seminar on Industry Perspectives** | |
| | Session Chair: David Scharp, MD, *Novocell* | |
| 12:00 – 12:30 | *Lunch* | |
| 12:30 – 12:45 | Host Response to the Islet Sheet, a Macroscopic Bioartificial Pancreas | Rick Storrs, PhD<br>*Islet Sheets Medical* |
| 12:45 – 1:00 | Alginate for Encapsulation: Functionality, Purity, Standards and Regulatory Considerations | Michael Dornish, PhD<br>*Pronovo Biomedical* |
| 1:00 – 1:15 | Factors Affecting the Transplant Efficacy of Islets Microencapsulated with Purified Alginate | Will Bachalo, PhD<br>*Islet Technology* |
| 1:15 – 1:30 | MicroEncapsulation of Islets for the Treatment of Type I Diabetes | Ingrid Stuiver, PhD<br>*MicroIslet* |
| 1:30 – 1:45 | Questions, Answers, and Audience Discussion | |
| | **Session IV: Immunoprotective and Anti-Inflammatory Therapies** | |
| | Session Chair: Ray Rajotte, PhD, *University of Alberta* | |
| 1:45 – 2:00 | Cellular Immunity to Immunoisolation Transplants | Ron Gill, PhD<br>*Barbara Davis Center for Childhood Diabetes* |
| 2:00 – 2:15 | Alginate Microencapsulation: Enhanced Islet Viability and Co-Encapsulation with Sertoli Cells | Ray Rajotte, PhD<br>*University of Alberta* |
| 2:15 – 2:30 | Peptide Blocking of Apoptosis to Preserve Beta Cell Function | Christophe Bonny, PhD<br>*University of Lausanne* |
| 2:30 – 3:10 | Questions, Answers, and Audience Discussion | |
| 3:10 – 3:40 | *Break* | |
| | **Session V: Gene Therapy for Islet Immunoprotection** | |
| | Session Chair: Bernard Thorens, PhD, *University of Lausanne* | |
| 3:40 – 3:55 | Systematic Engineering of Transplantable Beta Cell Line Using Lentiviral Transfer | Bernard Thorens, PhD<br>*University of Lausanne* |
| 3:55 – 4:10 | Adeno- or Lentiviral Vectors to Transfer Protective Genes into Isolated Islets | Massimo Trucco, MD<br>*Children's Hospital of Pittsburgh* |

| | | |
|---|---|---|
| 4:10 – 4:25 | Immunomodulation of Beta Cells in Transgenic Mice Using Adenovirus E3 Genes | Shimon Efrat, PhD<br>*Tel Aviv University* |
| 4:25 – 5:05 | Questions, Answers, and Audience Discussion | |

**Session VI: Discussion of Research Priorities**

Session Chair: David Klonoff, MD, *University of California – San Francisco*

| | | |
|---|---|---|
| 5:05 – 6:00 | Concluding Remarks | TBD |

**Day 2 — Friday, December 7**

**Working Session: Developing Workshop Recommendations**

| | |
|---|---|
| 8:00 – 11:45 | A select working group, including sponsors and session chairs, will meet to review the workshop's findings and translate them into recommended research priorities for future RFAs. |

# Appendix B: Workshop Participants

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