The bioartificial endocrine pancreas: overview.

J.P. Hallé
5415 boul. L'Assomption, Maisonneuve-Rosemont Research Centre,
Université de Montréal, Montréal, Canada (hallejp@videotron.ca).

Introduction

The term bioartificial endocrine pancreas refers to the transplantation insulin-producing ß-cells comprised in the islets of Langerhans (islets; the biological component) that are immuno-protected within semipermeable microcapsules (the artificial component) (Lim, 1980). Type 1 (insulin-dependent) diabetes is caused by an auto-immune destruction of over 90% of the pancreatic ß-cells. Current diabetes treatments improve the blood glucose control and delay but do not prevent the long-term complications of diabetes (DCCT 1993). In the non diabetic individual, there are minute-to-minute adjustments of the insulin secretion rate, in order to respond to the insulin need, which is continuously varying depending upon several parameters. Such continuous adjustment of drug dosing is usually not required for most diseases. Therefore, a treatment based on cell therapy is particularly well suited in this application. The transplantation of (non encapsulated) islets, using the Edmonton protocol, has induced insulin independence and normalized the 24h blood glucose profile in type 1 diabetic patients (Shapiro 2000), but it requires the life-long use of toxic immunosuppressive drugs. Therefore, this treatment is usually restricted to patients with an advanced and very unstable disease. In this application, the main objective of microencapsulation is to allow transplantation without the need to use immunosuppressive drugs, while avoiding the destruction of transplanted islets by allo or xenogeneic rejection or the autoimmune reaction. The use of microencapsulation in cell therapy presents special challenges as compared to industrial and ex vivo medical applications (e.g. bioreactors), particularly due to the critical issues of the biocompatibility of the method with the transplant and the host.

Concept

Immuno-isolation of transplanted cell in a perm-selective membrane has been and remains the basic concept of immunoprotection by microencapsulation. With this concept, the microcapsule plays a rather passive role. Nevertheless, this role may be complemented by active immuno-modulation. Since this effect is local, it still would be advantageous and safer than systemic immunosuppression. An example of such an approach is the temporary release of dexamethasone (or eventually other molecules) by microcapsule to overcome the effects of the implantation procedure (Bünger 2005). Alternatively or complementarily, the microcapsule surface could be coated by immuno-modulating molecules.

Research strategies.

The ultimate overall evaluation of the micro-device bioperformance is transplantation into a diabetic animal and evaluation of the effect on blood glucose. These outcome studies provide a picture of the state of the technology development at a given point. However, the progress of the technology requires the identification of the barriers to successful application and the in-dept understanding of the mechanisms that are involved. Techniques have been developed and detailed studies have been conducted to better understand and to quantitatively or qualitatively assess the microcapsule structure, characteristics and functions, as well as to investigate the mechanisms involved in the biocompatibility of the microcapsule with the transplant and the host.

Assessment of microcapsule characteristics and functions.

Methods have been developed to assess and modulate different microcapsule features, such as size, perm-selectivity, resistance to mechanical and chemical stresses and biocompatibility with the transplant and with the host. In recent years, high performance physico-chemical technologies, including nanotechnologies, have been used to obtain a better understanding of the microcapsule structure at the atomic and molecular scale (de Vos 202, Tam 2005, 2006). In particular, they were used to better understand the surface chemistry of the capsule surface (Tam 2005).

The mechanisms of the microcapsule immunogenicity

This includes immunogenicity of the biomaterials and of the whole microcapsules. Alginate remains the most widely used polymer for immobilising cells and forming the microcapsule core. Other polymers have been experimented. We (and others) have focussed our research in better understanding and improving the alginate-poly-L-lysine-alginate system.

Alginate is a natural polymer that is extracted from marine algae. As such it tends to be contaminated and is chemically heterogeneous. Many studies have been conducted on alginate purity (Dusseault 2005) and composition, particularly on the guluronic/mannuronic acid ratio (de Vos 2003). By optimizing both parameters, de Vos and his group have been able to obtain microcapsules that induce minimal cell overgrowth for as long as 2 years post implantation (de Vos 2003). However, the fact that other laboratories have not always been able to reproduce these results indicates a need for better standardization, which requires better understanding of underlying mechanisms. The work of the COST 865 project is an important collaborative effort to achieve these objectives. Three important types of alginate contaminants play a role in alginate immunogenicity: polyphenols, endotoxins and proteins (Dusseault 2005). These contaminants are abundant in nature, are present in crude alginates and have been shown to have a deleterious effect on alginate biocompatibility (Dusseault 2005). In an effort to identify other potential contaminants, we applied a non biased technique, X-Ray Photoelectron Spectroscopy (XPS), and we found no significant unknown contaminants (Tam 2006).

The mechanisms of immunogenicity of contaminants may be their release into the recipient and direct triggering of the immune system. Contaminants may also increase the immunogenicity by changing the physico-chemical properties of alginate, such as viscosity, hydrophilicity and interactions with the polycations (Tam 2006). The mechanisms underlying the effect of the alginate composition (G/M ratio) seem to be related to alginate interaction with the polycation rather than the effect on alginate itself (de Vos 2003, Tam 2006).

Studies of microcapsule true surface

In collaboration with the École Polytechnique de Montréal, we have combined tree complementary surface sensitive technology to improve our understanding of the capsule surface chemistry (Table 1) (Tam 2005).

Technique	Abbreviation	Information provided	Analytical Depth
X-Ray Photoelectron Spectroscopy	XPS	Quantitative analysis, Elemental composition, Chemical bonds	20 - 100 Å
Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy	ATR-FTIR	Three-dimensional conformation of proteins, Molecular structure, Binding behaviour	0.2 - 3 μm
Time-of-Flight Secondary Ion Mass Spectrometry	ToF-SIMS	Chemical composition of outer monolayer, Lateral distribution of molecules (imaging)	1 - 2 nm

Table 1. High performance technologies used for physico-chemical chemical analysis of capsule surface.

The usual theoretical model of APA microcapsules is a microcapsule composed of tree sheets: an alginate core, a semipermeable PLL layer and an outer coating of alginate. Since PLL was known to be immunogenic, the final incubation in diluted alginate aimed at completely covering and masking PLL. Nevertheless, our results showed that PLL represents approximately 80% of the APA capsule membrane and is present at the true surface of microcapsule i.e. in the 1-2 nm most superficial layers. This surface also contains larger complexes that are likely to be alginate-PLL complexes. Much less Na Alginate was found near the membrane. In addition, the presence intra-membrane PLL in the random coil conformation suggests that some PLL molecules may have a weak interaction with alginate. Unbound amine groups of the exposed PLL are likely to play a role in the immunogenicity of APA microcapsule. This knowledge is critical for developing methods to improve and assess the reproducibility of microcapsule biocompatibility.

Biocompatibility with the transplant: encapsulated islet cell survival.

Issues related to encapsulated islet cell viability include the biocompatibility of the biomaterials with the transplant (encapsulated cells), the mechanisms of cell death and survival, the physicochemical characteristics of the matrix, cell-extracellular matrix-polymer interactions, oxygen transport from the recipient blood vessels to the capsule and within the capsule to the islets. The implantation site plays a critical role as well. In the first decades of islet microencapsulation research, a much larger number of encapsulated than non encapsulated islets has been required to normalize blood glucose in diabetic animals. In contrast, a recent study (Korbutt 2003) demonstrated a better *in vitro* and *in vivo* survival of islets immobilized in alginate beads than free islets. PLL may affect islet viability depending upon PLL concentration and capsule size. Interventions have been designed to improve islet survival, such as the incorporation of oxygen transporters (Chae et al. 2004) and oxygen generators (Wu 1999) in the capsule matrix.

We (Robitaille 2003) have incubated islets in IGF-II for 24h before and 24 h after encapsulation and have significantly decreased cell apoptosis and necrosis and considerably improved *in vitro* and *in vivo* islet survival. The blood glucose was normalized in 2-fold more diabetic mouse recipients using 50% encapsulated islets incubated in IGF-II, compared to non IGF-II incubated islets. Using the same model, we recently showed that co-encapsulating islets and IGF-II producing bioengineered cells promotes *in vitro* and *in vivo* islet survival (manuscript submitted).

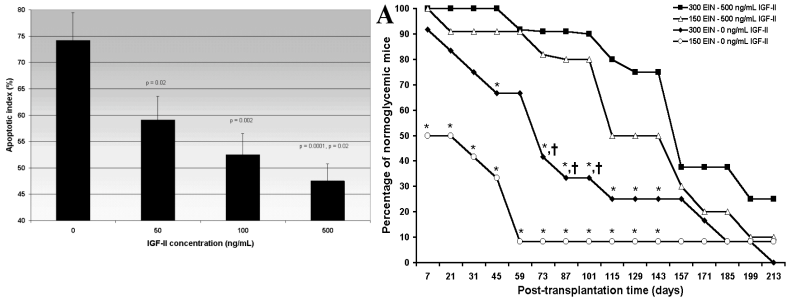

Fig 1 A. Effect of IGF-II on islet apoptosis in vitro. B. Effect of IGF-II on in vivo islet survival. Robitaille 2003.

Microcapsules with covalently cross-linked membranes

Microcapsule resistance to mechanical and chemical stresses is another important parameter, since a limited number of broken microcapsules may cause the release of immunogenic materials and trigger an immune reaction that could compromise the whole transplant. Different approaches have been experimented to improve the microcapsule stability, such as the use of multiple coatings, decrease of capsule size, increase of the polycation concentration and/or incubation time and photodimerization of the semipermeable poly-L-lysine sheet.
We (Leblond 2006, Dusseault 2005) have developed a method to induce the formation of covalent cross-links between the molecules of the polycation sheet and between the latter and alginates from both the core bead and the outer coating. The challenge was that most techniques that are use to create covalent links are not compatible with cell viability. However, using a heterobifunctional photoactivatable cross-linker, we were able to induce the formation of covalent cross-links without any harmful effect on *in vitro* and *in vivo* islet cell survival. The covalently cross-linked microcapsules were shown to be 22-fold more resistant to a standardized mechanical stress than standard capsules. Moreover, they remained intact after 3 years of incubation in a strong alkaline buffer (pH=12), whereas standard microcapsules dissolved within 45 seconds in the same conditions. The method has no deleterious effects on microcapsule permeability and encapsulated cell survival (Dusseault 2005, Leblond 2006).

A new role for novel microcapsules.

Using these extremely resistant microcapsules, we proposed a new role for micro-encapsulation: in addition to protect the transplant (islets) from the host immune system, they protect the host from the transplant, particularly from the risks of malignant cell transformation and dissemination of immortalized cell line and stem cell derived islets. As a proof of concept, very malignant EL-4 thymoma cells that were encapsulated in covalently cross-linked or standard microcapsules or non encapsulated were implanted intraperitoneally in mice. All recipients of ≥20,000 non encapsulated EL-4 cells died with widespread metastasis within a period of 26.5 ± 0.9 days. When 250,000 EL-4 cells encapsulated in standard microcapsules were implanted, the average survival time of recipients was slightly prolonged to 35.2 ± 2.2 days. The key finding was that all mice that were implanted with 250,000 EL-4 cells encapsulated within covalently cross-linked microcapsules were still alive with no sign of disease at 150 days post-implantation. This *in vivo* experiment confirmed the high strength of covalently cross-linked microcapsules. Moreover, it supports that concept the these strong microcapsules prevent the dissemination of malignant cells and therefore would improve the safety of transplanting cells with a potential of malignant transformation, such as stem cells, bioengineering cells or immortalized cell lines (see S6-5 for more details).

Perspectives. The progress in the development of a bioartificial endocrine pancreas requires that fundamental issues are addressed. Such issues include in-dept understanding of the microcapsule structure at the atomic and molecular scale, the chemistry of the microcapsule surface, which interacts with the host, the mechanisms of the host-surface

interactions, the identification of the host cells, molecules, and signaling pathways that are involved in the host reaction to the transplant, as well as the parameters affecting islet cell survival. Strategies must be developed to improve the microdevice biocompatibility with the host and the transplant and to promote encapsulated islet cell survival. The increased knowledge will allow to designing microcapsules with an optimal bioperformance. Critical to this endeavor are collaborative efforts between laboratories and a multidisciplinary approach. Ideally, this multidisciplinary effort should involve biomedical scientists, including biologists, physiologists, endocrinologists, experts in molecular and cell biology, biochemists and immunologists as well as experts from the engineering and natural sciences field, such as polymer scientists, biomedical engineering, material scientists and physicists.

References

Bünger CM et al. (2005). Deletion of the tissue response against alginate PLL by temporally release of co-encapsulated steroids. Biomaterials26: 2353-60

Chae SY et al. (2004). *Prolonged glucose normalization of streptozotocin-induced diabetic mice by transplantation of rat islets coencapsulated with crosslinked hemoglobin*. Transplantation. 78(3):392-7.

DCCT (The diabetes control and complications trial research group) (1993). *The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus*. N Engl J Med 329:977-986

de Vos et al. (2002). *Chemistry and biocompatibility of alginate-PLL capsules for immunoprotection of mammalian cells*. Biomed Mater Res 60: 252–259.

de Vos et al. (2003). *Long-term biocompatibility, chemistry, and function of microencapsulated pancreatic islets*. Biomaterials 24 (2003) 305–312

Dusseault J et al. (2005) *Microencapsulation of living cells in semi-permeable membranes with covalently cross-linked layers*. Biomaterials 26(13):1515-22.

Dusseault J et al. (2006) Evaluation of alginate purification methods: effect on polyphenols, endotoxin and protein contamination. J Biomed Mat Res A. 76(2):243-51.

Juste S et al. (2005) *Effect of poly-L-lysine coating on macrophage activation by alginate-based microcapsules: assessment using a new in vitro method*. J Biomed Mater Res A 72(4):389-98.

Korbutt G.S. et al. (2003*). Improved survival of microencapsulated islets during in vitro culture and enhanced metabolic function following transplantation*. Biomaterials 24:305–312

Leblond F et al. (2006) *Semi-permeable multi-layered microcapsule with covalently linked layers and method for producing the same*. patent U.S. Patent # 7,128,931. 2006.

Lim F et al. (1980) *Microencapsulated islets as bioartificial endocrine pancreas.* Science 210(4472):908-10.

Pueyo ME et al. (1993) *In vitro activation of human macrophages by alginate-polylysine microcapsules*. J Biomater Sci Polym Ed 5(3):197-203.

Robitaille et al. (2003). *Insulin-like growth factor II allows prolonged blood glucose normalization with a reduced islet cell mass transplantation*. Endocrinology 144(7):3037-45.

Shapiro AM et al. (2000) *Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen*. N Engl J Med 343(4):230-8.

Shapiro et al. (2006). *International trial of the Edmonton protocol for islet transplantation*. J Engl J Med 355:1318-30

Tam S et al. (2005). *Physicochemical model of alginate-poly-l-lysine microcapsules defined at the micrometric/nanometric scale using ATR-FTIR, XPS, and ToF-SIMS.* Biomaterials. 26:6950-61

Tam SK et al. (2006) *Impact of residual contamination on the biofunctional properties of purified alginates used for cell encapsulation.* Biomaterials 27:1296-305.

Thu B et al. (1996) *Alginate polycation microcapsules. I. Interaction between alginate and polycation*. Biomaterials 17(10):1031-40.

Wu et al. (1999). *In situ electrochemical oxygen generation with an immunoisolation device. Ann N Y Acad Sci*. 875:105-25.