Biomedicine Handbook

Mark Walters

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Edited by **Mark Walters**

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Preface

The world is advancing at a fast pace like never before. Therefore, the need is to keep up with the latest developments. This book was an idea that came to fruition when the specialists in the area realized the need to coordinate together and document essential themes in the subject. That's when I was requested to be the editor. Editing this book has been an honour as it brings together diverse authors researching on different streams of the field. The book collates essential materials contributed by veterans in the area which can be utilized by students and researchers alike.

This book compiles advanced researches in the field of biomedicine. Complete and methodical technical researches in cell biology and molecular biology have promoted the enhancement of traditional medicine. Experts are now able to understand a few puzzling medical difficulties due to the stress on molecular levels. This book concentrates on the advancements in biomedical science dealing with regenerative medicine, gene medicine and medical instruments. This book is a compilation of numerous researches done by experts and will be beneficial for readers interested in this field.

Each chapter is a sole-standing publication that reflects each author´s interpretation. Thus, the book displays a multi-facetted picture of our current understanding of application, resources and aspects of the field. I would like to thank the contributors of this book and my family for their endless support.

Editor

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Regenerative Medicine

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Encapsulation and Surface Engineering of Pancreatic Islets: Advances and Challenges

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1. Introduction

Type 1 diabetes (T1D) is a chronic autoimmune disease representing a major health care problem worldwide (Tierney et al., 2002). T1D is caused by islet-reactive immune T cells that destroy insulin-producing pancreatic β-cells. Transplantation of insulin-producing pancreatic islets by their injection in vascularized organs has been recently recognized as a promising path to curing diabetes (Meloche, 2007; Robertson, 2000). However, despite the significant promise, the clinical application of the procedure remains limited due to (a) limited supply of islets suitable for transplantation, (b) a hypoxia because of a low tension of oxygen at the implantation sites and (c) an acute rejection during transplantation. One of the challenges is associated with isolation and culturing islets *in vitro* before injection. In the pancreas, endocrine cells of the islet clusters are separated from exocrine cells by a discontinuous mantle of collagen fibers defining their respective basement membrane. During collagenase isolation of islets from the pancreas, further disruption of the islet mantle results in preparations exhibiting various morphological changes (islet fragmentation, fusion) under routine tissue culture conditions, particularly in human islets (Lacy & Kostianovsky, 1967). Attenuation of islet viability and functionality accompanies these morphological changes. The second issue is associated with islet transplantation which requires immunosuppression to protect the donor islets from the host immune response and prevent implant rejection and post-surgery inflammations (Ricordi & Strom, 2004). Despite the fact that a range of immunosuppressive drugs have demonstrated pharmacologically inhibitory effects on pro-inflammatory cytokines (Riachy et al., 2002; Contreras et al., 2002; Lv et al., 2008; Stosic-Grujicic et al., 2001), the use of immunosuppressive molecules is very specific since they can induce non-specific suppression of the immune system resulting in serious side effects and increased risk of infection which can work against the benefits of a transplant (Narang & Mahato, 2006). These issues have inspired the development of a number of strategies to prevent immunogenic reactions and stabilize islet morphology and functionality, both *in vitro* and following transplantation *in vivo* (Chandy et al., 1999; Abalovich et al., 2001). Two major approaches have been introduced to prevent immunogenic reactions on the islet surfaces: macro and microencapsulation of the islet cells and islet cell surface modification (Fig. 1) (De Vos et al., 2003; Panza et al., 2000; Scott & Murad, 1998; Opara et al., 2010).

Islet macro/microencapsulation strategy is based on embedding islets in solid matrices, allowing for the creation of a semi-permeable environment around islets capable of immune-protection and for mass and oxygen transfer (Beck et al., 2007; Weber et al., 2007). For that, the isolated islets are usually entrapped individually or as islet clusters in thick gels, for example, high-viscous alginate droplets stabilized with divalent ions of barium or calcium (Zimmermann et al., 2001). Islet surface modification strategy involves covalent conjugation of molecules to islet cell surfaces. However, this technology is limited to the introduction of specified functional small molecules to cells and might interfere with cell physiology (Rabuka et al., 2008; Paulick et al., 2007). Layer-by-layer (LbL) technique has been recently applied as a new approach to modify islet surfaces (Krol et al., 2006; Wilson et al., 2008). The technique is based on alternating LbL deposition of water soluble polymers on surfaces from aqueous solutions which results in nano-thin coatings of controllable thickness and composition (Decher & Schlenoff, 2002; Kharlampieva & Sukhishvili, 2006; Tang et al., 2006).

Unlike bulk encapsulating materials, the ultrathin conformal coating affords a faster response to stimulation and the possibility to bind factors or protective molecules to the protective ultrathin shell with the later slow triggered release of these molecules (Chluba et al., 2001). By selecting specific pairs of polyelectrolytes, a defined cutoff of the coating (Kozlovskaya & Sukhishvili, 2006) is possible, as is inhibitor binding to prevent graft rejection, microphage attacks, or antibody recognition (Kim & Park, 2006). Here, we review methods and devices designed for protecting isolated islets from host immune responses while allowing transport of essential nutrients. We also discuss challenges of various approaches developed for encapsulation of individual islets in thin coatings that conform to the islet surfaces, fabricated using a number of physical and chemical processes.

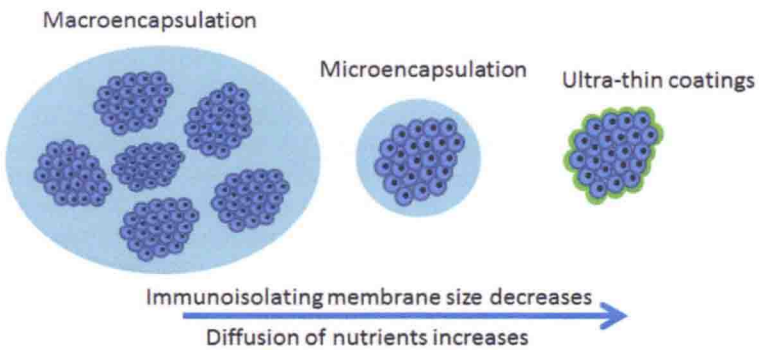

Fig. 1. Strategies for encapsulation and surface engineering of pancreatic islets.

2. Preservation of islets *in vitro*

Recently, the advantages of cultured islets before transplantation have been demonstrated over freshly isolated islets (Herring et al., 2004; Ichii et al., 2007). It is known that cellular stress due to pancreas preservation and islet isolation process leads to a loss of islets during the first 24 h after isolation. Islet culture after isolation can prevent islet cells from toxic

factors generated by cells damaged during these processes, providing sufficient oxygen and nutrients to allow islet cells to recover. After isolation of islets from donors, it is crucial to maintain islet viability and functionality until transplantation to give sufficient time to perform microbiological tests as well as donor matching and recipient pre-conditioning.

Modifying the islet preparations for reducing immunogenicity by altering temperature (Kim et al., 2005; Stein et al., 1994), or media composition is one of the advantages for islet pre-culture (Ricordi et al., 1987; Murdoch et al., 2004). For example, supplementation of culture media with lactogen hormones has been shown to minimize β-cell loss during pre-transplant culture leading to a higher β-cell survival rather than proliferation (Yamamoto et al., 2010; Nielsen, 1982). When islets were cultured in media supplied with recombinant human prolactin (rhPRL) for 48 h, production of interferon-gamma (IFN-γ), tissue necrosis factor-alpha (TNF-α), interleukins cytokines, IL-6, IL-8 and microphage inflammatory protein-1-β was comparable with the control group of islets with no increase in pro-inflammatory mediators in the presence of rhPRL suggesting no elevated immunogenicity. Furthermore, the PRL treatment of islet preparations resulted in decreased apoptosis in β-cell subsets, suggesting β-cell specific anti-apoptotic effects of rhPRL (Yamamoto et al., 2010). Another possible issue with the pre-cultured islets is the possibility of islet fusion during incubation, which may lead to hypoxia and starving of the cells. Those result in central necrosis of fused islet aggregates causing a significant loss of islet potency and viability (Ichii et al., 2007).

Apoptosis of human islets after isolation from supporting extracellular matrix is a very common cell pathway *in vitro*. During the first steps towards apoptosis integrin expression is diminished and, consequently, phenotype characteristics are lost and islets stop secreting insulin (Ris et al., 2002). Exploring the parameters important for preventing pre-apoptotic events should help in preserving islet viability and function for long periods of time. The effects of two types of collagen, type I and type IV, and fibronectin, proteins that are generally present in the cell-supporting matrix have been explored (Daoud et al., 2010). Islets have a tendency to spread and form a monolayer on surfaces *in vitro*. The islet monolayer can still be viable without preserving the phenotype characteristics, however, the normal insulin secretion of islets will be lost. Daoud et al. showed that integrity and insulin production of islets can be preserved by presence of fibronectin in the medium (Daoud et al., 2010). Both types of collagen increased the viability of islets from 24 to 48 hours *in vitro*. Several studies revealed an increasing survival of islets *in vitro* when embedded in a solid matrix. Culture in collagen I gels obtained from rat tail and fibrin gels have shown promising for prolonging islet survival (Wang & Rosenberg, 1999; Beattie et al., 2002).

3. Approaches to prevent immunogenic reactions on the islet surfaces

The immune reactions against encapsulated islets can be divided into non-specific immune activation initiated by surgery; a host response against the encapsulating materials and implanted islets provokes the immune response by releasing the bioactive molecules.

The instant blood-mediated inflammatory reaction (IBMIR) is an inflammatory reaction that occurs when isolated islets come in contact with human blood. This process is responsible for islets destruction together with overall failing of transplantation. One of the major

triggers for the IBMIR reaction is a secretion of tissue factors by islets surfaces. Disruption of host tissue by surgery leads to release of bioactive molecules such as fibrinogen, histamine and fibronectin. Release of serum components and presence of extracellular matrix and cell debris attract tissue macrophages to the surgery site to clean up and start the process of wound healing. The immune cells have the ability to produce various small bioactive compounds such as interleukins, tissue necrosis factors (TNF) and histamine. There are several extravascular approaches to prevent IBMIR reaction against pancreatic islets (Nafea et al., 2011).

3.1 Macro and microencapsulation

'Macroencapsulation' can be defined as encapsulation of large numbers of islets together in one device. The shape of the devices can be a hollow fiber, planar membranes or macrocapsules. Macroencapsulation provides immune-isolation of islets within semi-permeable membranes. The major advantage of macrocapsules is the possibility to easily retrieve the islets from an implantation site in case of surgery complications. Capsules reduce the risk of the IBMIR reaction from occuring, therefore, allowing the use of not only allogenic but also xenogenic materials and avoiding the use of immunosuppressive medications. However, macroencapsulation has not found a broad use in islet encapsulation and transplantation due to a large volume of protective devices. Relatively large sizes will cause limited oxygen and nutrition access and, as a result, cell necrosis (De Vos et al., 1999). Such macrocapsules do not allow for tuning the molecular weight cutoff (or semi-permeable properties) to prevent recognition by antibodies, and cytokines cannot be sufficiently excluded either (Cui et al., 2004). Similarly, recently developed poly(ethylene glycol) (PEG) hydrogels although demonstrated facile control over porosity but formed microbeads are large and present a barrier for rapid molecular transport (Weber et al., 2007). The capsules with larger diameters than an islet itself are also expected to plug blood vessels. This can exert harmful effects on the patient's liver. The diameter of encapsulated islets must be much smaller than that currently attained to allow transplantation of the islets into portal veins. Thus, new methods for the microencapsulation of islets without increasing the diameter of the implant are required. In this respect, modification of islet surfaces would be a powerful tool that can provide an artificial nurturing environment and preserve islet viability and function (Raymond et al., 2004; Wilson & Chaikof, 2008; Ricordi & Strom, 2004; Lim et al., 2011).

In contrast to macroencapsulation, 'microencapsulation' can be defined as encapsulation of single islets or a small group of islets inside the polymer gel coating. Microencapsulation can be achieved via the formation of a gel shell around the islets by polymerization of a precursor solution around islet surfaces. Emulsification is one of the microbead formation methods (Iwata et al., 1992; Yang et al., 1994). PEG/Alginate was used for islets microencapsulation through their mixing with two-phase aqueous emulsion. The islets contained in emulsion microdroplets underwent cross-linking with calcium ions (Calafiore et al., 2006).

During the microencapsulation process, a bio-inert coating with minimal host response and cell toxicity should be created. Individual islet coating offers a number of advantages over the macroencapsulation. An individual coating provides a better surface:volume ratio that allows faster diffusion of oxygen and nutrients which supports the viability of encapsulated

islets. Another advantage is the possibility to employ different coating techniques. The major requirement for the materials used for islets encapsulation is biocompatibility which can be evaluated by a degree of fibrotic overgrowth (Liu et al., 2010). Overgrowth of fibrous tissue upon microcapsule surface would affect the oxygen and nutrition diffusion by clogging microcapsule pores (Nafea et al., 2011). Indeed, insufficient supply of oxygen is the major reason for necrosis of microencapsulated islets. The biocompatibility of the materials used for microcapsules formation strongly depends on chemical composition of materials and applied purification techniques.

Also, biocompatible materials must have selective permeability to promote the survival of encapsulated islets. Selective permeability should allow fluxes of oxygen, nutrients and metabolism products freely in and out of the protective membrane. At the same time the microcoating should prevent the immune system compounds such as antibodies and cytokines to reach the encapsulated cells. The viability and functioning of encapsulated islets is in majority determined by the molecular weight cutoff of the microcapsules. The cutoff determines the upper size limit of molecular weight that is allowed to go through coating. However, diffusion of the molecules very often depends on size, shape and charge of the molecules. Diffusion coefficient and permeability can be considered the more useful and informative characteristics of the cutoff. Control over the permeability can be achieved by varying polymer molecular weight, increasing or decreasing functional group and cross-linking densities and polymerization conditions (Dembczynski & Jankowski, 2001). Molecular weight and concentration of polymer can drastically change the permeability of the hydrogel. It has been shown that poly(vinyl alcohol) (PVA) hydrogel with a high molecular weight of PVA backbone had a higher swelling ratio due to the decrease in cross-link density (Martens et al., 2007). In radical polymerization, an increase in polymer concentration leads to the increase in cross-link density and decrease in the swelling ability. The chemical structure of hydrogel has a significant effect on hydrogel permeability. Presence of electrostatic interactions between charged hydrogel groups and small molecules will slow down the diffusion rates over time. Moreover, during time, changes in the diffusion coefficient occur because of changes in the hydrogel network due to physical or chemical absorption of proteins.

The microcapsule shape (Sakai et al., 2006) morphology such as roughness (Bunger et al., 2003), mechanical properties and especially stiffness of the hydrogel (Berg et al., 2004), play an important role in provoking immune response or fibrotic overgrowths. The importance of hydrogel roughness was demonstrated for implanted rough poly(L-lysine) (PLL)/Alginate microcapsules and only smoothing the surface by adding poly(acrylic acid) completely abolished the tissue response (Bünger et al., 2003). In most cases, smooth round surfaces had the lowest fibrosis promoting effect (Zhang et al., 2008). The most commonly applied materials for microencapsulation are alginate (Lim et al., 1980), agarose (Iwata et al., 1992), PEG (Weber & Anseth, 2008) and poly(hydroxyethylmetacrylate-methyl methacrylate) (Dawson et al., 1987).

3.1.1 Alginate microgels

Alginate microcapsule production can be made under physiological conditions and provide an environment that allows maintaining the islets functionality and viability.

The microcapsule fabrication technique is based on the entrapment of islets within the spherical droplets that are produced by extrusion of solution containing polymer and islets through a needle. Two forces are usually used to control the size of droplets: an air flow that builds around the tip of the needle (Wolters et al., 1992), and a high voltage pulse around the needle tip (Halle et al., 1994; Hsu et al., 1994), the formed droplets very often require an additional stabilization via gelation and beads formation (Stabler et al., 2001). Cross-linking with metal ions, calcium and barium, and chemical or covalent cross-linking are the two methods generally used in microbead or microcapsule formation. Calcium cross-linked alginate microcapsules very often require an additional stabilization with PLL.

Alginate is a linear polysaccharide extracted from algae with a chemical structure combining different ratios of α-L-guluronic (G) and β-D-mannuronic acids (M) and saccharides (Haug et al., 1974). Chemical composition of alginate may affect the biocompatibility (De Vos et al., 1997) or/and function, and the activity of incorporated cells (Stabler et al., 2001). Guluronic acids and alternating mannuronic-guluronic acids have a high ability to cooperatively bind with divalent metal ions and form a cross-linked gel. Barium ion cross-linked gels have a higher stability compared to calcium ion cross-linked gels, though barium ions produce a more stable cross-linking only for alginate with a high ratio of guluronic acid (> 60%). However, ionically cross-linked alginate hydrogels undergo slow degradation under physiological conditions. During this process, alginate microcapsules with metal ion cross-linking undergo slow exchange of divalent cations with sodium ions, which leads to the microgel degradation. Leakage of barium ions is not desirable due to their toxicity which is based on their ability to inhibit K^+ channels at concentration greater than 5-10 mM (Zimmermann et al., 2000). Variations in alginate structures obtained from different alginate sources introduce an additional limitation for the use of the barium cations for alginate cross-linking. Permeability and swelling of alginate-based capsules also strongly depend on the ratio of the acids in alginate. Barium cross-linked G-rich alginate beads had lower permeability to IgG than Ca^{2+} cross-linked microgel. However, cross-linking M-rich alginate beads with Ba^{2+} ions leads to the permeability increase and an overall higher gel swelling (Mørch et al., 2006). Applying an outer coating of polycations such as PLL, or poly-L-ornithine can readily stabilize the alginate capsules with low G-ratio (Thu et al., 1996). Thus, the alginate must be chosen according to a specific application. Moreover, polycations, such as PLL, are proinflammatory molecules responsible for the fibrotic overgrowth (King et al., 2001) and the soluble PLL induces the cytokine production in monocytes and can be a reason for cellular necrosis (Strand et al., 2001).

3.1.2 Poly(ethylene glycol)-based gels

PEG is a less immunogenic material that is generally studied for use in islet encapsulation. PEG is a non-ionic hydrophilic polymer stable at physiological conditions with the highly hydrated polymer coils. The variation in its molecular weight can be used to control protein adsorption and the permeability of PEG gels by changing their porosity unlike alginate (Chen et al., 1998). However, the formed microbeads are larger and diffusion of molecules is slower (Weber et al., 2007a).

Selective-withdrawal coating technique was used to encapsulate rat pancreatic islets within PEG thin coating (Wyman et al., 2007). Geometry of the capsules is determined by selective withdrawal throughout the bulk solution and allows the cross-linking throughout the