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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.

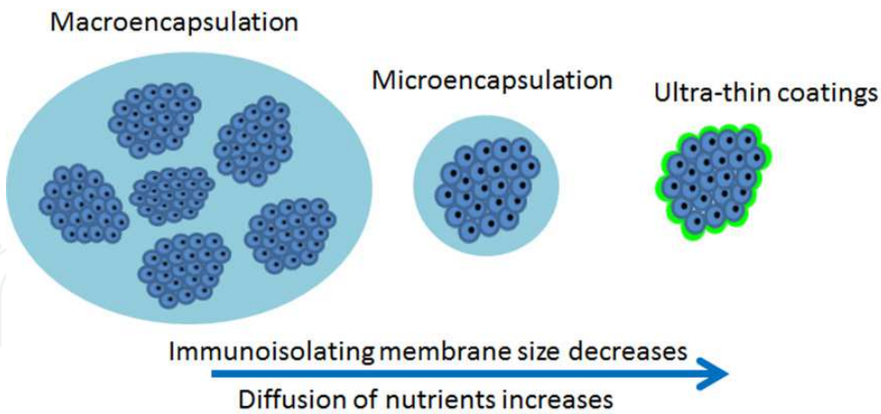

Fig. 1. Strategies for encapsulation and surface engineering of pancreatic islets.

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

volume of the formed capsules. Mixture, containing PEG diacrylate, photoinitator accelerator and islets were mixed in a chamber. Once the islets were trapped into droplets, they were drawn into the spout. PEG polymerization was done by the exposure of the droplets to a green light. The free-radical polymerization of PEG diacrylate produce branched polymer chains. The encapsulated islets passed through the withdrawing tube with the same inner diameter that allowed for the same size capsules. Islets encapsulation required two encapsulation rounds. Despite this double coating produced capsules with mean thickness of 20.5 μm, islets were capable to respond to high glucose stimulation without a delay.

Hydrogel network diffusion properties can be adjusted by altering the cross-link density or polymer molecular weight. The PEG microgel cross-linked by photoinitiated polymerization is a well studied system for islet encapsulation. An average pore size of the PEG gel formed by chain polymerization is dependent on the length of the polymer chains between cross-links and can be used to predict and calculate the diffusion coefficient within the gel. Studies revealed that the molecular weight of PEG can not only affect the cross-link density, but also can change the concentration profile of radicals in time. Changes in the concentration of free radicals affect both a number of cross-linkable double bonds and diffusion limitation introduced during gel formation (Weber et al., 2008). More importantly, microcapsules produced from PEG with M_w 2000–10,000 g/mol via photopolymerization did not affect the viability of encapsulated islets or alter their insulin secretion. The level of glucose secretion observed during 1 hour of glucose stimulation was at the same level as for unmodified islets. Insulin secretion from islets encapsulated into 1 mm thick PEG gel was delayed at specific time point within stimulation period. The diffusion-related delay can be minimized through reducing the distance between encapsulated islets and surrounding environment by reducing the thickness of the gel (Weber et al., 2008).

The created hydrogel environment is extremely different from the natural surroundings of islets. Indeed, cell viability is strongly dependent on cell-cell or cell-matrix interactions. For example, the viability of MIN6 pancreatic β-cells encapsulated in just PEG-based matrix reduced to 17% within 10 days (Weber et al., 2007b). Mimicking the extracellular environment in an artificial matrix can provide additional enhancement of islets viability and functioning. For that, adhesive peptides or various biological components for promoting cell-matrix interactions can be co-polymerized within hydrogels. Thus, physical incorporation of laminin and collagen type IV, the most abundant proteins present in the extracellular matrix, was performed during formation of hydrogel from PEG dimethacrylate block co-polymers via photoinitiated polymerization (Weber and Anseth, 2008; Weber et al., 2006). Such introduction of the proteins to the PEG matrix preserved the viability and functioning of the microencapsulated islets up to 28 days in culture. Interestingly, the ratio of the proteins had an effect on the level of insulin secretion from the encapsulated islets. For example, equal amounts of laminin and collagen IV in the hydrogel did not have any synergetic effect on the insulin production, while enhanced insulin secretion was observed for the increasing amount of laminin.

Isolation and purification of matrix proteins is, however, complex and cost inefficient way to recreate extracellular matrix environment. The substitution of natural proteins by artificial sequences is a promising approach to resolve this issue. Recently, several therapeutic agents (Drucker, 2001; Drucker, 2002; Holz & Chepurny, 2003) have been proposed to increase

insulinotropic effect of pancreatic cells and protect them from apoptosis. The most promising agent is glucagon-like peptide 1 (GLP-1) that has an extremely short half-life time (less than 2 min) *in vivo* (Lee et al., 2005). Synthesis of artificial peptides with the same biological activities as a substitution for the natural peptide can be one of the solutions for this problem. Synthesis and evaluation of the synthetic glucagon like peptide functionality had been made by Lin et al (Lin & Anseth, 2009). The biological activity of the synthetic peptide physically entrapped in photopolymerized PEG microgel was not affected by photopolymerization. Presence of the synthetic substitute of GLP-1 in the hydrogel had a positive effect on insulin secretion and viability of rat islets encapsulated into the PEG hydrogel.

Many cytotoxic molecules of a low molecular weight are able to easily diffuse inside the microgel capsules *in vivo*. Early islet graft failing and islet damaging occurs due to infiltration of pro-inflammatory cytokines, such as interleukin-1β (IL-1β), tissue necrosis factor-alfa (TNF-α), and interferon-gamma (IFN-γ). The size of these molecules allows them to freely diffuse within the matrixes commonly used in islets encapsulation. A reasonable approach to resolve this issue would be a further reduction of microcapsule permeability. However, the microcapsule pore size cannot be reduced much and still has to allow the permeability of insulin. The decrease in capsule size generally leads to a higher permeability towards cytotoxic molecules. For example, the islets encapsulated into 400-500 μm capsules had minor damages from cytokines, while the islets encapsulated into capsules with smaller dimensions become more susceptible to damage by cytokines (Basta et al., 2004). Moreover, a combination of cytokines and their concentration are also important factors (King et al., 2000; De Vos et al., 2003a).

Incorporation of molecules that can locally inhibit interactions between the small cytotoxic molecules and islets can be used to increase the life-time of encapsulated islets. Indeed, when anti-inflammatory peptide, the cytokine-inhibitory peptide IL-1RIP, had been introduced into PEG hydrogel even in low molar ratio, the survival of islets challenged with the combination of three pro-inflammatory cytokines (IL-1β, TNF-α and IFN-γ) reached up to 21 days with 75 % of cells surviving while less than 25% cells survived by day 4 in the absence of the anti-inflammatory peptide (Su et al., 2010). Furthermore, a synergetic protective effect had been observed for the IL-1RIP within the microgel, with even slightly enhanced insulin secretion in the presence of IL-1RIP in the microgel. Importantly, the hydrogel was produced by mixing 4-arm PEG macromers modified with cysteine and 4-arm PEG with thiol-end groups. The formed hydrogel network possessed the thiol groups that exhibited mild reductive properties and could protect encapsulated cells from oxidative stress (Robertson & Harmon, 2007; Pi et al., 2007).

Immobilization of cytokine antagonizing antibody within the hydrogel is complicated due to the large size of such a molecule (antibodies are usually of hundreds kDa), and protein stability and functioning can be altered during the immobilization process. Additionally, the presence of foreign antibodies in microgel structures can provoke host immune system response. Employing small molecules with the similar function can be one of the solutions such an issue. For example, small WP9QY peptide has been identified as a potential antagonist peptide that binds to human TNF-α. This peptide was used to produce the TNF-α-antagonizing PEG hydrogel (Lin et al., 2009). When the PEG hydrogel was formed via photoinitiated polymerization in presence of acryl-WP9QY and adhesive peptides in a

precursor solution, encapsulated islets had higher insulin secretion even in the presence of TNF-α.

3.1.3 Cryopreservation of islets using microencapsulation

One of the common applications of microgel beads is cryopreservation of islets. Cryopreservation can be achieved through freezing to preserve the cells within the polymer matrix. However, during freezing islets can still be damaged by extracellular ice formation and cell shrinkage due to dehydration nature of freezing. Recently proposed vitrification approach allowed avoiding these issues. In this process, the presence of certain compounds, e.g., sugars (Doxastakis et al., 2005), stabilizes cells through a direct substitution of water molecules in the outer hydrophilic part of the membrane which leads to the formation of a glass that prevents fusion and reduces dehydration-induced stresses (Koster et al., 2000). Cryopreservation by freezing had been shown to have a significant negative effect on the structure of agarose gel microcapsules (Agudelo & Iwata, 2008). Cryopreservation changed the molecular network structure of agarose gel. Structural changes affected the diffusion of molecules within the gel. The small molecules, such as glucose, were still able to freely diffuse through porous gel, while, molecules of a medium size, such as insulin, had much lower diffusion coefficient after gel freezing. In contrast, vitrification process applied to the same materials had not introduced any changes in scaffolds. Islets preserved by vitrification do not suffer from ice formation and shrinkage and islets viability is drastically increased. Rat pancreatic islets were successfully cryopreserved by freezing in alginate beads (Schneider & Klein, 2011) or in poly(vinyl alcohol) macro-scaffolds (Qi et al., 2010). In the former case, islets were mixed with alginate solution of ultra-high viscosity prior to freezing. Beads then were formed using an air jet droplet generator to create a certain flow rate and finally cross-linked with barium ions. After 7 days of cryopreservation islets were able to maintain normal function *in vitro* and normalized non-fasting blood glucose in diabetic mice *in vivo*. However, since vitrification procedure requires the presence of low molecular weight molecules, some of them, such as poly(vinyl pyrrolidone) and PEG of a low molecular weight, can exert some toxicity to the vitrification solution. For example, insulin release ability of the islets vitrified in the presence of those compounds decreased by 45% after a vitrification/warming cycle (Agudelo & Iwata, 2008).

3.1.4 Encapsulation in epoxy-based microcontainers

New approach for islets microencapsulation has been introduced by Gimi et al. (Fig. 2) (Gimi et al., 2009). They proposed to use microcontainers made by polymerization of epoxy-based polymer. The microcontainer has a membrane containing nano-slots with the size of 25 nm that permit the bidirectional transport of metabolites and nutritional ingredients. Highly cross-linked epoxy-based polymer SU-8 with high glass transition temperature, thermal stability and high Young's modulus was used in fabrication of the microcontainer structure. The containers are capable of holding islets with dimensions upto $200x200x200\mu m^3$. Overall device consists of two major components: a hollow cubical base and a nano-porous lid. The cubical base is intended for islets encapsulation, and the lid is sealed after an islet is placed inside the base. The base and the lid have a similar multilayer structure and a complicated forming procedure involving metal sputtering, polymer coating and nano-slot fabrication steps. Murine islets encapsulated into the microcontainers were viable after 48 hours in culture. The main advantage of this microcontainer approach is the

possibility to directly visualize the encapsulated islets due to optical transparency of the microcontainer material. However, apart from complex fabrication, this approach might require additional steps for biocompatibility adjustment of the material and needs additional studies on islet long-term survival and functioning.

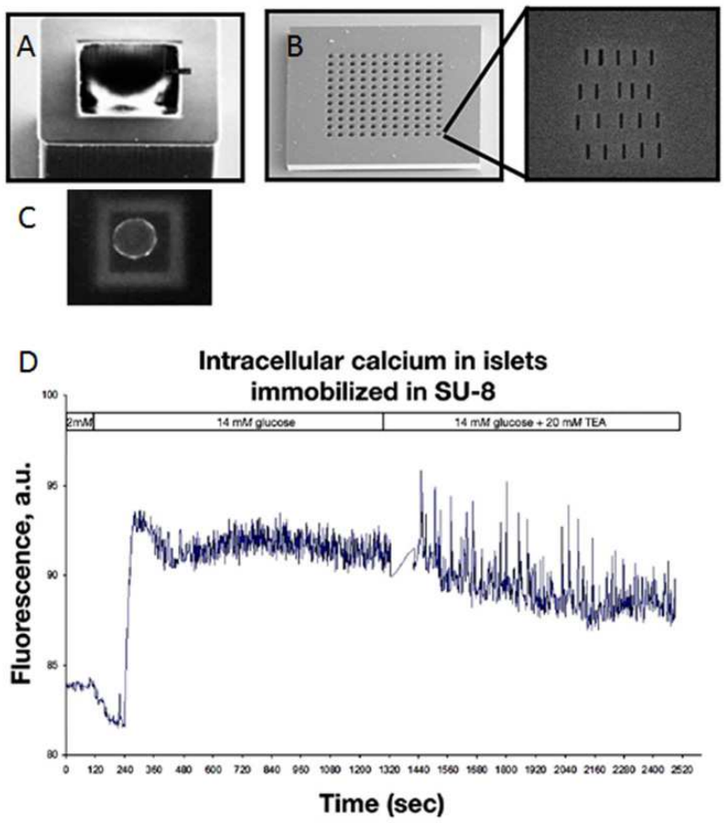

Fig. 2. Scanning electron microscope images of the microcontainer base (A) and lid (B) showing the 500-nm thin-membrane island structures. The thin membrane recessed within each island was milled with 25-nm slots using a focused ion beam. For encapsulated islets, the intracellular Ca^{2+} changes of an encapsulated islet in response to glucose and tetraethylammonium stimulations were measured using a spectral two-photon confocal microscope (C), and changes in the concentration of intracellular Ca^{2+} in response to glucose and tetraethylammonium were quantified as seen in this representative trace (D). Reprinted with permission from Gimi, et al., 2009 with permission from Journal of Diabetes Science and Technology.

3.2 Surface modification

Surface modification of living cells is a new approach in islet encapsulation. The main idea of surface modification is to reduce the size of implanted islets. This can be achieved by forming a conformal protective layer on the islet surfaces using various surface modification techniques. Both synthetic and natural polymers can be used to introduce various functional groups on the islets surfaces. Covalent binding to amino groups of cell membrane proteins, insertion of amphiphilic polymers into a cell membrane via

hydrophobic interactions between a lipid bilayer of the cell membrane are general approaches employed in cell surface modification (Fig. 3). The covalent binding of protective layer to islet cell surfaces is expected to be stable and be present at the islet surface for certain periods of time.

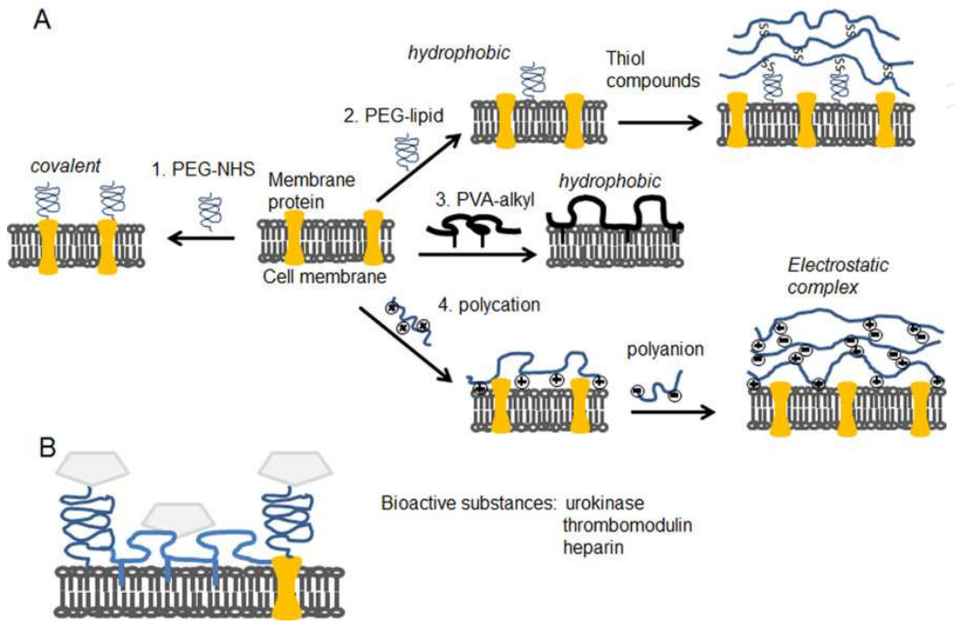

Fig. 3. (A) Modification of cell surfaces by covalent conjugation to cell surfaces (1), by incorporation of molecules into the cell membrane via hydrophobic interactions (2, 3), via electrostatic interactions of polyions with the cell membrane (4). Immobilization of bioactive molecules to cell surfaces via interactions of the cell membrane with polymers (B). Adapted from Teramura & Iwata, 2010 with permission from Elsevier.

3.2.1 Covalent conjugation of molecules to islet surfaces

Thin conformal coatings based on PEG are highly attractive for cell surface modification due to its biocompatibility and protein-resistant properties based on PEG low interfacial free energy with water, its unique properties in aqueous solutions, its high surface mobility, and its substantial steric stabilization effects (Amiji & Park, 1993). PEGylation has been used for camouflaging islet surfaces to immunologically protect transplanted islets from the immune system (Panza et al., 2000; Lee et al., 2002; Lee et al., 2004). Methoxy-PEG-succinimidyl propionic acid molecules conjugated with amino groups of collagen matrix at the islet surface through a stable amide bond protected islets from immune cell attack (Fig. 3, A1). The conjugated PEG molecules have been shown to block immune cells from diffusing to the transplanted islets, which allowed islets to function in the diabetic recipients for several weeks (Lee et al., 2006a; Lee et al., 2006b; Lee et al., 2007). Another advantage of the nano-thin PEG layer is the small size of the produced shell compared to the size of encapsulated islets. The small shell size and the demonstrated protection against the host immune system should allow transplantation into the portal vein by catheter injection and still prevent immune response of the host.

To achieve grafting of the end-functionalized PEG polymer onto the islet surfaces, islets are cultured in media in the presence of various concentrations of the polymer. To increase the amount of a grafted polymer on surfaces, the incubation time can be increased or the incubation step can be repeated. The grafting of methoxy-PEG (mPEG) onto the islets surface is usually carried out via the succinimidyl ester end groups introduced in mPEG. These groups couple to amino groups present in the collagen matrix around islets. Controlling the grafting time is important since longer grafting times can lead to diffusion of PEG inside the islets which may increase a chance of swelling and exposure of islets to outside environment. Furthermore, long grafting time increases the probability of islet damaging (Lee et al., 2002).

The molecular weight of the grafted PEG is another important parameter for islet surface modification. Barani et al., showed that grafted mPEG of 5 and 10 kDa onto islets isolated from Wistar rats had a different effect on cell functioning and viability (Barani et al., 2010). Insulin secretion for the islets grafted with 5 kDa mPEG was at the same level as for unmodified islets, while overall insulin secretion from islets modified with 10 kDa mPEG decreased. However, its protective ability was higher which correlates well with the decrease in mesh size and thus less exposure to immune system. The effect of PEG molecular weight was also studied on porcine islets (Xie et al., 2005). The highest protection ability without affecting islets functional capability was found for 5-6 kDa PEG that allowed complete coverage of islets surface.

In the case of two reactive succinimidyl ester end groups, additional functional molecules such as albumin can be brought to the islet surfaces (Xie et al., 2005). Introducing albumin and PEG on the surface can be used to suppress the immunogenic reactions (Hortin et al., 1997). Presence of human albumin not only increased cytoprotection of islets but also significantly increased the insulin production which was possible due to increasing density of protective coverage with albumin presence. The so-modified islets maintained their functionality *in vivo* up to 15 days.

Heparin is a highly sulfated glycosaminoglycan and is very often used as an injectable anticoagulant. Systemic delivery of heparin at therapeutic doses, however, substantially increases the risk of bleeding. Furthermore, the effect of soluble heparin is limited to 2-3 hours *in vivo* and has no significant effect on long-term IBMIR reaction (Bennet et al., 1999). At the same time, heparin, immobilized on artificial surfaces which mimic the protective ability of the endothelial cells, demonstrated inhibition of coagulation and complement activation (Bennet et al., 1999). Immobilized heparin coating was successfully made on human, porcine and mouse islets by step-by-step incubation procedure (Cabric et al., 2007). Islet surfaces were first biotinylated through covalent attachment of succinimidyl ester-biotin, then incubated with avidin and finally, heparin conjugate was covalently attached on the modified islets surface (Fig. 3, B). The heparin coating produced by this method was present of the islets surfaces at least 72 hours, though not detectable after 4-5 weeks of transplantation.

Islets surface modification with other inhibitors of the coagulation cascade through bifunctional PEG linkers can provide another strategy to reduce IBMIR. Human recombinant thrombomodulin was conjugated to the islet surface through bifunctional PEG grafts (Stabler et al., 2007). Thrombomodulin is an endothelial cell transmembrane protein

which can deactivate procoagulant–proinflammatory properties of thrombin through its binding (Esmon et al., 2004). The formed thrombomodulin–thrombin complex leads to rapid inactivation of clotting factors Va and VIIIa and reduction of new thrombin generation (Esmon et al., 1982). This approach of thrombomodulin conjugation allowed for the substantial delay of clot formation upon incubation of islets in human plasma with preserved islet viability and glucose-stimulated insulin secretion capability.

Despite the immunoprotective capabilities, the camouflaging layer of PEG cannot provide a long-term protection against small cytotoxic molecules produced by the immune system in response to metabolic activity of encapsulated islets after the transplantation. A combination of a protective coating and a low dose of an immunosuppressive drug can be a way to provide a suppression of host immune system without causing highly toxic effects. A low dose of Cyclosporine A has been shown to effectively prevent rejection of PEG-modified islets and support their functioning and survival up to 1 year (Lee at al., 2007). Similar effect was demonstrated by employing 6-arm-PEG-catechol that allowed for a coating with a higher density and a lower amount of immunosuppressive drug Tacrolimus (Jeong et al., 2011). Catechol is a surface independent anchor molecule which ensured conjugation of PEG-catechol to collagen matrix around the islet. The survival time of 6-arm-PEG-catechol grafted islets was similar to that of unmodified islets. However, the administration of the drug increased the survival time of 6-arm-PEG-catechol grafted islet almost two times.

3.2.2 Incorporation of molecules in the cell membrane

Islets surface modification can be achieved via hydrophobic interactions of amphiphilic polymers, such as PEG-phospholipids and PVA with long alkyl chains, with lipids in cell membranes (Takemoto et al., 2011; Chen et al., 2011). Presence of hydrophobic parts, phospholipids and alkyl chains, in polymer and cell membranes are responsible for spontaneous incorporation of co-polymers into the lipid layers of cell membrane. The spontaneous incorporation is greatly affected by the length and hydrophobicity of alkyl chains (Rabuka et al., 2008; Teramura et al., 2007). Using this approach, the PEG-phospholipid-based coating was modified with fibrinolytical enzyme urokinase and with a soluble domain of the anticoagulant, thrombomodulin (Fig. 3, B) (Chen et al., 2011). The immobilization of heparin, urokinase, or thrombomodulin can improve the graft survival after the transplantation. The maleimide-PEG-lipids were utilized to immobilize proteins on the islets surface through reaction of maleimide and thiol-modified proteins. However, thiol groups can easily form disulfide bonds under physiological conditions and that decreased the efficiency of the conjugation.

Urokinase is a serine protease that activates plasminogen into plasmin, which dissolves fibrin blood clots. Urokinase can be conjugated onto the surface of islets to dissolve blood clots surrounding islets in the liver for inhibition of the cascade reactions (Takemoto et al., 2011). Hybridization between urokinase and DNA-PEG-lipids was made due to complementary sequences on the protein and single strain DNA (Fig. 4). DNA hybridization method is versatile for conjugation of various bioactive molecules. Using different sequences, it was possible to conjugate different molecules on the cell surfaces. The cell morphology was not affected by such modification, although, urokinase activity disappeared after 4 days in culture.

Overall, surface modification achieved via hydrophobic interaction and insertion of hydrophobic chains into the cell membrane demonstrated a limited retention time. For PEG-lipids this time depends on the alkyl chain length of lipid chains (Inui et al., 2010). The longer alkyl chains allowed PEG-lipid molecules to retain longer on the cell membrane. However, the overall retention time was no longer than 48 hours before the all molecules disappeared from the islets surfaces. Incorporation of hydrophobic molecules into the cell membrane leads to their uptake into the cell cytoplasm. Adding amphiphilic part to the structures prevents the uptake but molecules are released into the surrounding media.

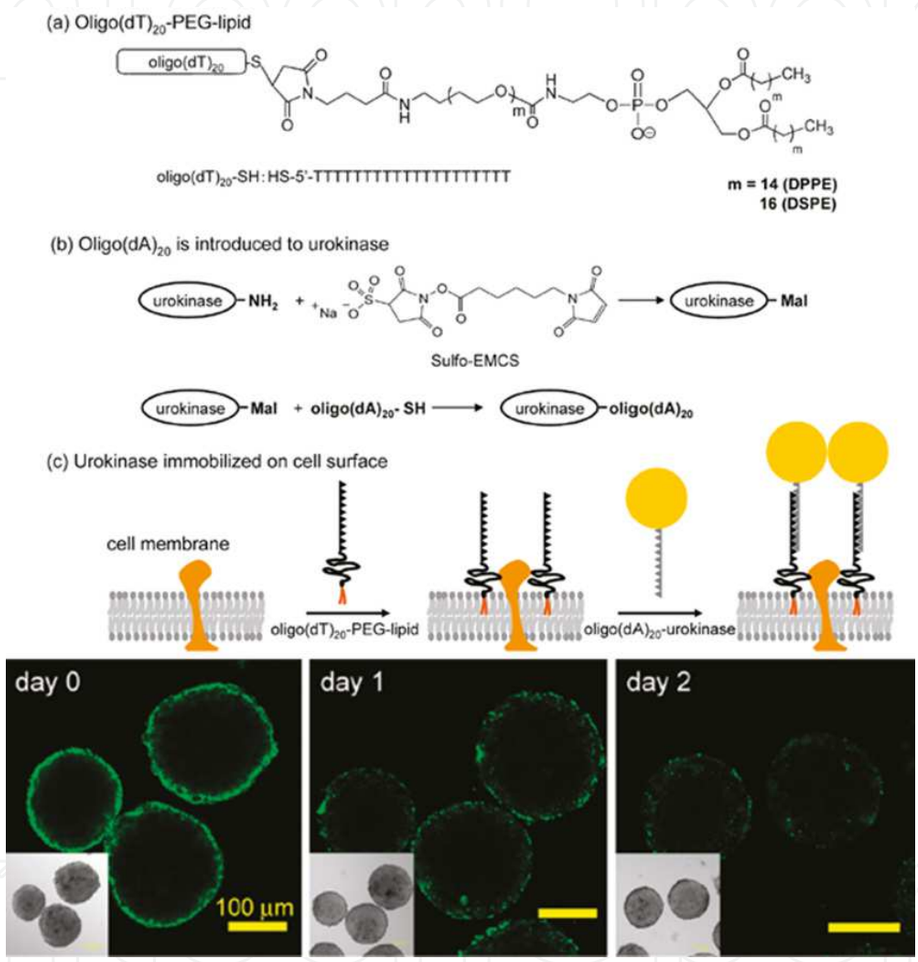

Fig. 4. Top: (a) Chemical structure of oligo(dT)$_{20}$-conjugated PEG-lipid (oligo(dT)$_{20}$-PEG-lipid). (b) Urokinase is modified with Sulfo-EMCS to introduce maleimide groups, and then oligo(dA)$_{20}$-SH is conjugated on urokinase through the thiol/maleimide reaction.
(c) Oligo(dT)$_{20}$-PEG-lipid is incorporated into the cell surface by the hydrophobic interaction between alkyl chains and the lipid bilayer of the cell membrane. Oligo(dA)$_{20}$-urokinase is applied to cells carrying oligo(dT)$_{20}$. Urokinase is conjugated on the cell surface through oligo(dT)$_{20}$ -oligo(dA)$_{20}$ hybridization. Bottom: Confocal laser scanning microscopic images of urokinase--modified-islets which were subjected to immunostaining for urokinase. Islets were modified with oligo(dT)$_{20}$-PEG-lipids and then oligo(dA)$_{20}$-urokinase. The urokinase-modified-islets just after treatment, after 1 and 2 days of culture. Reprinted with permission from Takemoto et al., 2011. Copyright 2011 American Chemical Society.

3.2.3 Surface modification through electrostatic interactions

Heparin has an affinity to a number of plasma proteins and growth factors including endothelium and vascular endothelium growth factors. Employing this property of heparin, the synthetic heparin-binding peptide amphiphile was used to immobilize heparin and, subsequently, growth factors on islet surfaces (Chow et al., 2010). Initial immobilization of the amphiphile on the islet surfaces can be achieved *via* electrostatic interactions of positively charged amphiphile and negatively charge cell membrane (Fig. 3, A4). Later, negatively charged heparin can be bound to the amphiphile. The so-formed amphiphile-heparin complex coating on the islets surface was able to bind and retain growth factors up to 48 hours. The complex nanostructures provide extracellular matrix-like scaffold for the encapsulated islets for supporting their viability. Such immobilization of growth factors does not affect viability or function, yet, they are able to stimulate an angiogenic response in the islets.

3.2.4 Immobilization of living cells on islet surfaces

Endothelial cells on the islets surfaces have a good tolerance towards blood presence and can provide a protection against IBMIR. Introduction of such protective cells around islets can be used as an effective protection strategy. The human aortic endothelial cells were introduced onto isolated human islets of Langerhans by mixing of both cell types and incubation for several hours (Johansson et al., 2005). The clotting can be significantly reduced with the 90%-cell-coating present on islet surfaces. The consistency of the cell amounts in such coating can be improved using the PEG-phospholipid-based approach (Teramura & Iwata, 2009; Teramura & Iwata, 2008a). In that case, islets and human endoderm kidney cells were first separately biotinylated through biotin-PEG-lipid anchors to cell membranes with further streptavidin coating on the kidney cells (Fig. 5). Immobilization of the endothelium cells around islets was then made via streptavidin-biotin reaction. The cell enclosure was stable on the islet surfaces within 3-5 days *in vitro*. To overcome the streptavidin immunogenicity, the cell deposition was also made via the PolyDNA-PEG-lipid conjugate (Teramura & Chen, 2010; Teramura & Minch, 2010). Human endoderm kidney cells were able to rapidly proliferate forming a cell multilayer on the islets surfaces protecting the encapsulated islets from the host immune response. However, the cell oxygen consumption can result in lowered oxygen available for the encased islets. Thus, additional studies are necessary to clarify the short- and long-term effects of the cell presence on islets surfaces.

3.3 Conformal coating of islets

3.3.1 Layer-by-layer (LbL) approach

The layer-by-layer (LBL) assembly of polymers based on sequential adsorption of oppositely charged components is one of the established methods for the preparation of thin polyelectrolyte multilayer films with controlled properties. The LBL represents a universal surface modification approach that allows for producing surface-attached films with controlled thickness, permeability, mechanical properties and surface chemistry. The technique has been recently applied to modify islet surfaces (Krol et al., 2006; Wilson et al., 2008). The LbL modification of islet surfaces is based on alternating deposition of water

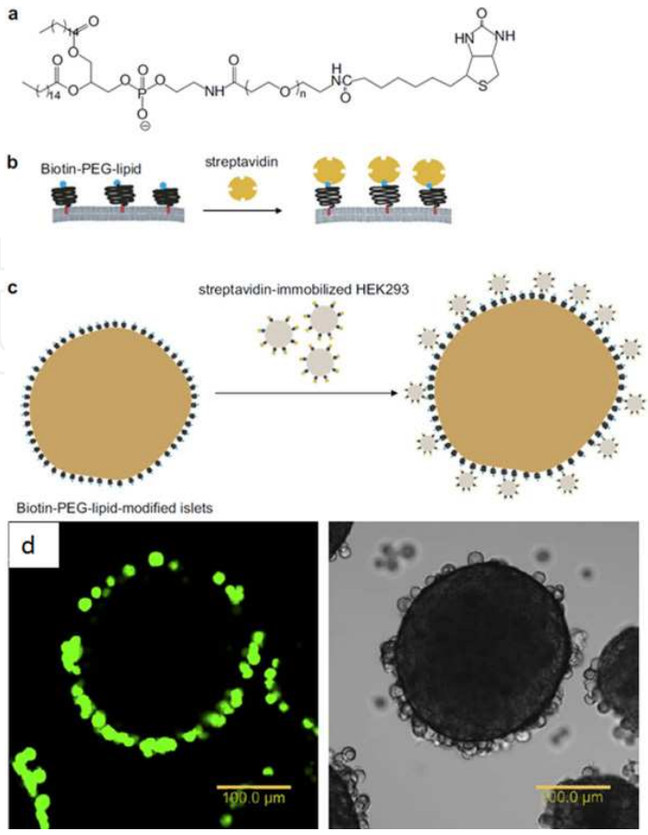

Fig. 5. (a) Chemical structure of biotin–PEG-conjugate (biotin–PEG–lipid). (b) Schematic illustration of the interaction between streptavidin and biotin–PEG–lipid at the lipid bilayer cell membrane. Biotin–PEG–lipid has hydrophobic acyl chains and is incorporated into the cell surface by anchoring into the lipid bilayer. Streptavidin is immobilized on the cell surface by anchoring to biotin–PEG–lipid. (c) Scheme for the immobilization of streptavidin-immobilized HEK293 cells on the surface of biotin–PEG–lipid-modified islets. After mixing streptavidin-immobilized HEK293 cells and biotin–PEG–lipid-modified islets, they were cultured in medium at 37°C on a culture dish. During culture, HEK293 cells were spread and grown on the cell surface to cover the whole surface. (d) Hamster islets modified with biotin–PEG–lipid and immobilized with streptavidin-immobilized HEK293 cells. The HEK293 cells were labeled with CellTracker. Reprinted from Teramura & Iwata, 2009 with permission from Elsevier.

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). 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 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). Modification of the last coating layer can be used to support functionality of islets and reduce the immune response from a host system. The cutoff of the polyelectrolyte multilayer (PEM) is defined by polyelectrolytes used in coating formation (Krol et al., 2006).

3.3.2 The ionic LbL assembly

To promote a multilayer film formation on the cell surfaces, the negatively charged cell surface is treated with a cationic polymer solution and the cell surface is further exposed to an anionic polymer solution to form an electrostatically-paired polyelectrolyte complex film (Fig. 3, A4). Effect of molecular weight of polyelectrolytes and the charge of outermost layer was demonstrated in case of the LBL encapsulation of human islets into poly(allylamine hydrochloride)/poly(styrenesulfonate sodium salt) (PAH)/(PSS) and poly-(diallyldimethylammonium chloride) (PDADMAC)/PSS layers. Islets encapsulated into PAH/PSS and PDADMAC/PSS multilayers using a higher polycation molecular weight demonstrated a limited insulin release due to a lowered permeability of insulin through the polyelectrolyte membrane. A decrease in a polycation molecular weight resulted in larger pores of the polyelectrolyte membrane and restored responsive relationship between glucose stimulation and insulin response of the coated islets (Krol et al., 2006).

Most cationic polymers widely used in the LbL modification of surfaces such as poly(L-lysine) (PLL) and poly(ethylene imine) (PEI) are extremely cytotoxic and cells treated with the polycations can be severely damaged. Their cytotoxic effect though has been observed to be dependent on polycation concentration and exposure time (De Koker et al., 2007). The overall cytotoxicity of the polyelectrolytes originates from positive charge of polycations which can induce pore formation within the cell membrane causing its damage and, eventually, cell death (Bieber et al., 2002; Godbey et al., 1999). The high toxicity of the PAH/PSS LbL film was confirmed by Wilson et al (Wilson et al., 2008). They demonstrated that coating the murine islets with only 3 layers of PAH/PSS/PAH led to the reduction of islet viability by 70%. Similar effect was found for islets coated with 3 layers of PLL/alginate LbL film. Even 15 minutes of islets incubation with low concentration of PLL results in ~60% decrease in cell viability. Menger et al showed that PLL was able to pass through the lipid bilayer if it was previously allowed to form complex with anionic lipids (Menger et al., 2003). PEI was found extremely toxic to the islets. This polycation destroys the cell membrane immediately after its interactions with the membrane surface (Teramura et al., 2008b). The overall charge arrangement of a polycation and its interaction with the cell membrane strongly depends on the three-dimensional structure and flexibility of the polymer chains. It has been shown that polymers with highly flexible chains and a high cationic density will exert tremendous cytotoxicity. Thus, the polycations with globular structures demonstrated good biocompatibility, whereas polymers with more linear and flexible structure such as PLL and PEI showed higher cytotoxicity (Teramura et al., 2008b).

Since the polycations toxicity partially depends on the polymer charge density, it can be attenuated by conjugating neutral molecules, such as PEG, to the critical number of amino groups along the polycation backbones. PEGylation of PLL is carried out through grafting of N-hydroxysuccinimide-PEG (NHS-PEG) chains to amino groups on PLL backbone to produce PLL-g-PEG. The grafted PEGs are unbranched, hydrophilic, discrete-length molecules in the form of Methyl-PEGn-NHS ester, where the subscript "n" denotes a number of the ethylene glycol units. The NHS ester end group is spontaneously reactive with primary amines, providing for efficient PEGylation of amine-containing molecules or surfaces. The methoxy(ethylene glycol) grafts were conjugated to PLL backbone through a covalent attachment to lysine residues (Wilson et al., 2009). Forty percent of PEG substitutes on the PLL chain allowed for attenuation of the PLL positive charges without any

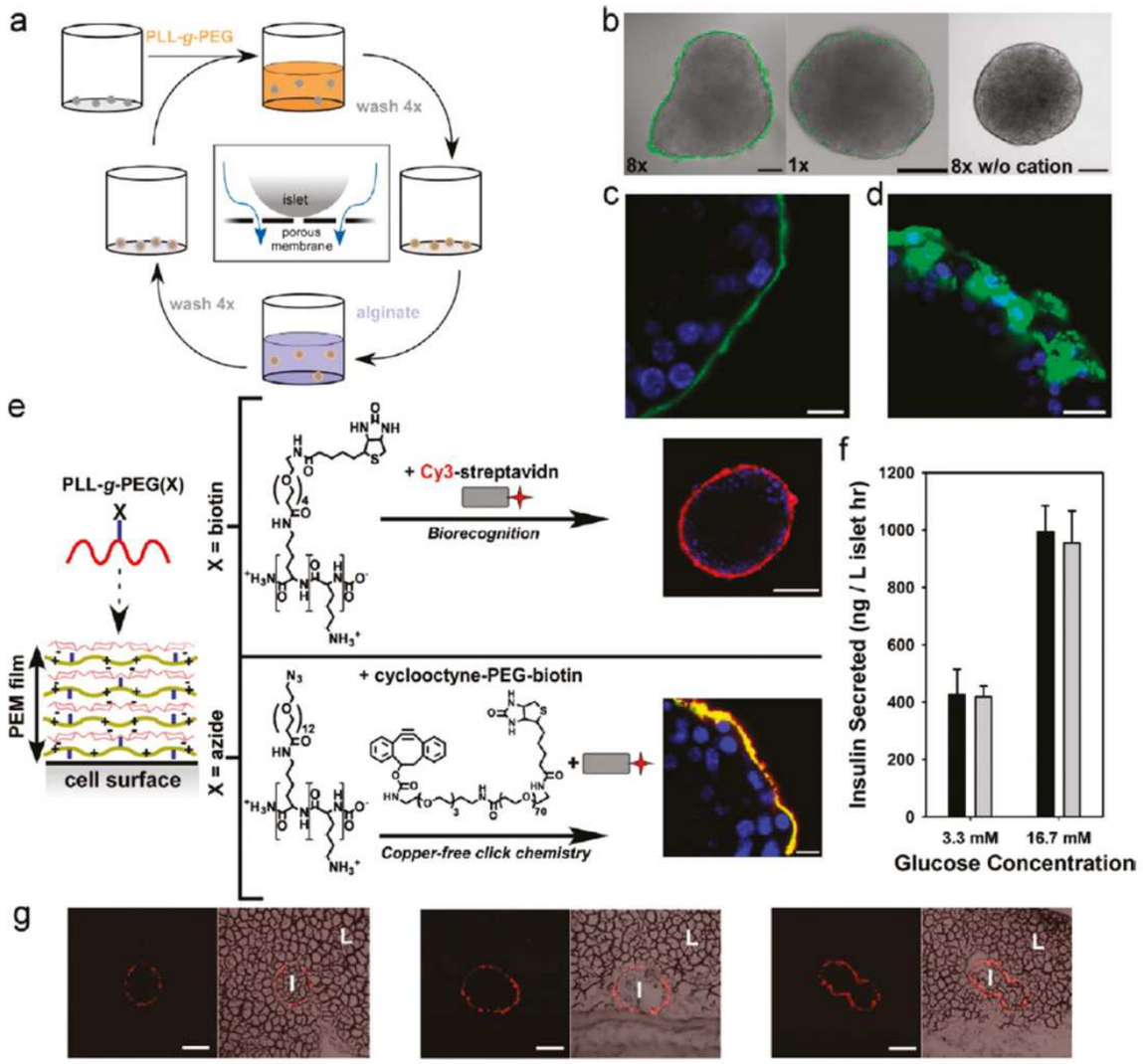

Fig. 6. Cell-surface-supported PEMs were assembled on individual pancreatic islets through LbL deposition of PLL-g-PEG copolymers and alginate. (a) Method to assemble PEMs on islets. (b) Representative confocal micrographs overlaid on bright-field images of coated islets coated using flourescein-labeled alginate (F-Alg) with eight bilayers (8x), a single bilayer (1x), or treated only with F-Alg (8x, w/o cation). (c) F-Alg is localized on the extracellular surface of cells, confirming the cell-surface-supported nature of films. (d) Deposition of a single PLL/F-Alg bilayer resulted in intracellular internalization of alginate by peripheral cells. (e) Chemistry and reactivity of cell-surface-supported films can be tailored through integration of biotin- and azide-functionalized PLL-g-PEG copolymers. (f) Insulin secretion by islets coated with a $(\text{PLL-g-PEG /alginate})_8$ film (gray) and untreated islets (black) in response to a step-change in glucose. (g) Confocal (left) overlaid on bright-field micrographs (right) of frozen sections of liver (L) after intraportal transplantation of islets (I) engineered with PEM films labeled with streptavidinCy3. Scale bars: b,e (top), g=50 μm; c,d,e (bottom)=10 μm. Reprinted with permission from Wilson et al., 2011. Copyright 2011 American Chemical Society.

deleterious effect on the islet viability (Fig. 6). The modification of PEG grafts with various functional molecules, such as biotin, hydrazide and azide, can extend the functional capabilities of the PLL-g-PEG-based LbL islet coatings. For example, the deposition of the PLL-g-PEG-biotin outmost layer on top of the PLL-g-PEG/Alginate multilayer film can generate surface densities of biotin functional groups comparable with that obtained by the treatment of islet surfaces with only NHS-PEG-biotin molecules (PEGylation/biotinylation through the NHS-ester coupling). Unlike the latter, the former approach is advantageous as it does not alter cell surface morphology and allows for controlled densities of the biotin on the modified islet surfaces (Krishnamurthy et al., 2010). The first successful *in vivo* transplantation of PEM engineered islets was demonstrated for the murine islets coated with conformal PLL-g-PEG/Alginate LbL films (Fig. 6g) (Wilson et al., 2011).

High toxicity of most polyelectrolyte polycations limits their use in biomedical applications. However, natural biopolymers chitosan and alginate have more similarities with the extracellular matrix, are chemically versatile and have a good biocompatibility. These linear polysaccharides carry opposite charge and can be electrostatically bound in a PEM. The $(\text{Chitosan/Alginate})_3$ islet coating was achieved *via* alternate deposition starting from positively charged chitosan (Zhi et al., 2010). The deposition conditions had been shown to greatly influence the islets viability, which well correlates with difference in charge density and toxicity of the polycation at high and neutral pH values. Additional protective outermost coating layer of phosphorylcholine (PC)-modified chondroitin-4-sulfate was introduced to reduce non-specific protein adsorption of the film. PC-moieties demonstrated remarkable repelling and hemocompatible properties. Increase in the coating thickness by adding additional layers of (Chitosan/Alginate) up to 5 bilayers did not adversely affect islets viability and insulin release, and the coated islets were viable up to 5 weeks of post-encapsulation.

3.3.3 The LbL assembly based on covalent and/or specific interactions

A multilayer PVA membrane formed via the layer-by-layer assembly was investigated for islet immunoprotective capabilities (Fig. 3, A3) (Teramura et al., 2007). In this approach maleimide-PEG-conjugated phospholipids were used to first modify the cell membrane surface to promote further interactions with PVA derivatives. It is well-known that PEG-conjugated phospholipids can be immobilized on the cell membrane through incorporation of the lipid chains into the cell membrane due to their hydrophobic interactions with the lipid bilayers of the membrane (Iwata et al., 1992). Moreover, PEG-phospholipids are more compatible with cells compared to polycations used in the ionic LbL surface modification of islets. A layer of PVA with introduced thiol groups (PVA-SH) was covalently attached to the maleimide-PEG anchors via thiol-maleimide reaction. The LbL multilayer of PVA was then deposited by alternating immersion of the islets into PVA-SH and PVA-pyridyl disulfide (PVA-PD). The driving force for the multilayer formation was a thiol/disulfide exchange reaction between the PVA derivatives. This ultra-thin PVA membrane affected neither cell viability nor insulin release function.

The LbL assembly of heparin multilayers was investigated for suppression of instant blood-mediated inflammatory reactions for the case when islets are to be transplanted through the portal vein to liver (Luan et al., 2011). The heparin well-known for its anti-thrombogenic properties was co-assembled with human soluble form complement receptor 1 (sCR1) which

is a potent inhibitor of the classical and alternative complement activation pathways (Pratt et al., 1996). The islets surfaces were first modified with PEG-phospholipid conjugates bearing maleimide groups (Fig. 7). The thiol-modified sCR1 molecules were then covalently bound to islets through the maleimide-PEG-lipid anchors via thiol-maleimide reaction. Following heparin immobilization with sCR1 was possible due to its strong affinity to sCR1. It is known though that covalent immobilization does not allow for long-term stability of the immobilized molecules at the cell surfaces due to fast degradation of the conjugation bonds. For instance, PEG-lipids gradually disappeared from the cell surface without uptake into the inside of cells dissociating from the cell surface into the medium (Teramura et al., 2008b). However, increase in the number of heparin /sCR1 layers upto 3 bilayers allowed subsequently increase the heparin and sCR1 retention time on islets surfaces up to 8 days *in vitro* with the gradual release from the islet surfaces over longer time periods. As the severe IBMIR reactions, especially the activation of the coagulation system and the complement cascade, occur in the first 2 days after transplantation, the heparin/sCR1 LBL approach can be a promising method for protecting the transplanted islets. Nevertheless, the protective effects and anti-thrombin activity of the LbL coatings are to be yet evaluated *in vivo*.

The LbL coating of islets based on streptavidin/biotin anchoring as a driving force for the LBL was also developed (Dai et al., 2007; Kizilel et al., 2010). PEGylated multilayers were grown using LbL assembly of biotinylated PLL and streptavidin. PEG was incorporated into the LbL architectures by assembly with biotin-derivatized PLL-g-PEG (Dai et al., 2007). In another example, the rat islet cell membranes were first biotinylated through covalent reaction of the membrane proteins and NHS-ester groups of NHS-PEG-biotin. The second layer of streptavidin was followed by a layer of a biotin-PEG conjugate with the insulinotropic Glucagon-like Ppetide 1 (GLP-1) (Kizilel et al., 2010). GLP-1 is a 30-amino acid peptide hormone produced in the intestinal epithelial endocrine L-cells. The main action of GLP-1 is to stimulate insulin secretion, acting as an incretin hormone, and to stimulate islet growth and neogenesis. Insulinotropic activity of GLP-1 is glucose-dependent and exerted via interaction with the GLP-1 receptor located on the cell membrane of the β-cells. Stimulation of insulin secretion due to GLP-1 immobilization at the islet surfaces can result in reducing the number of islets necessary for normalization of blood glucose of a patient. GLP1-PEG-Biotin was immobilized as an outermost layer to enhance insulin secretion. Islets coated with PEG-GLP-1 demonstrated enhanced insulin secretion and no time-delay in response to high glucose level. Streptavidin, however, is an immunogenic protein derived from bacteria *Streptomyces avidinii* and in most cases is not suitable for use in clinical studies.

Obviously, multilayer PEGylation technique provides an advantage over the single layer via enhancing immune-protective barrier but, on the other hand, the LbL method based on subsequent immersion of islets in alternating polymer solutions can be time-consuming and thus inappropriate for clinical use. The ultra-thin multilayer build-up process was further modified by Hume et al to overcome this issue (Hume et al., 2011). In their approach, the hydrogel was formed via photoinitiated polymerization of PEG diacrylate (PEGDA) or methacrylate pre-cursor solutions. Proteins such as immunoglobulins, and intercellular adhesion molecule-2 (ICAM-2) were immobilized on the coating surface via thiol-(meth)acrylate co-polymerization. The methacrylate-PEG was found to be more

cytocompatible compared to acrylate-PEG and demonstrated protective effects against T cells.

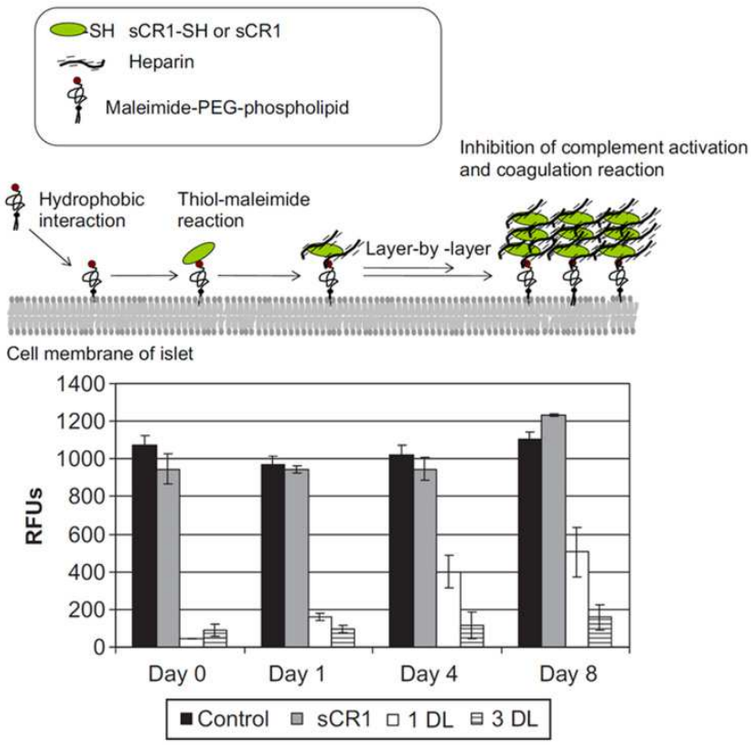

Fig. 7. Immobilization of sCR1and heparin on the islet cell surface (top). Relative thrombin inactivation activities of multiple sCR1-heparin layers (1 double-layer (1DL), three-double layer (3 DL) on glass plates. The activities were determined after the glass plates were maintained in culture medium (medium RPMI-1640 containing 10% FBS) for the indicated periods (bottom). Reprinted from Luan et al., 2011 with permission from Elsevier.

Thus, despite the advantages the ultrathin coating can present, chemical modification of the cell membrane using covalent conjugation of molecules, for example, covalent PEG binding via NHS-ester conjugation to the cell membrane proteins, can result in damaging of the membrane proteins and, consequently, in cell physiology disturbance. Moreover, the immunoisolation capabilities of ultra-thin hydrogel-based multilayers are still limited because of poor control over selective permeability towards small cytotoxic cytokines, and hypoxia within the gel environments. Current investigations are therefore focused on synthesis of hydrogels containing bioactive moieties, rather than unmodified coatings, aiming at "smart" protection of encapsulated islets from the damage originating from T-cells and small free radical species.

4. Outlook and perspective

Though pancreatic islet transplantation has emerged as a promising treatment for diabetes, its clinical application, however, remains limited due to serious side effects of immunosuppressive therapy necessary to prevent host rejection of transplanted islets.

Lifelong requirement of immunosuppressive drugs has deleterious effects on β-cell function and on host's ability to fight disease. To protect islets from immune-mediated destruction, camouflaging the islet surfaces is necessary for immunoisolation and immunoprotection. Current islet modification strategies are challenging for transplantation due to interference with islet functioning, limited nutrient transport, and/or cytotoxicity. Thus, current strategies mostly involve either imbedding islets within thick hydrogels that have limited nutrient transport and require large injection volumes or covalent conjugation to islet surfaces, which can interfere with cell function.

The ultra-thin coating approach possesses several advantages. Islets modified with such coatings can be easily implanted into liver through the portal vein, the preferable site for islet implantation. Islet necrosis can be avoided because of the rapid diffusion of nutrients and oxygen through the coating. Moreover, due to a minimal volume of the enclosed islets, an adequate release of insulin can be achieved in response to blood glucose changes. Though, despite the advantages, chemical modification of the cell membrane using covalent conjugation of molecules to the cell membrane proteins can result in cell physiology disturbance. In this respect, non-covalent modification method based on LbL can afford for cytocompatible coatings if non-toxic components are used. The ultrathin LbL coating allows for 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. By selecting specific polyelectrolytes, a defined cutoff of the coating is possible, as is inhibitor binding to prevent graft rejection, microphage attacks, or antibody recognition. Another important point is that the mechanical properties of the LbL-based films can be adjusted through an introduction of appropriate components or the chemical modification of the constituents. This issue is particularly important as β-cell in mature islets can still grow (Dor et al., 2004) causing the islet size increase. Unlike hydrogel-based ultrathin coatings, LbL coatings have the ability to withstand islet size increase and effectively enclose the islets for long periods of time. Another crucial issue is the preservation of the islet integrity because functionality losses were observed when islets disintegrated or fused in suspension culture. In this respect, the LbL technique provides a conformal and stable coating of individual islets.

However, despite the significant promise of the LbL strategy for islet modification, the main drawback of the approach is cytotoxicity of the used compounds. Moreover, ultra-thin encapsulating films may not work as reliable barriers against free-radicals. Indeed, thick agarose microbeads or alginate microcapsules can provide a more effective shield capable of inactivating free radicals. This issue is particularly crucial since most inflammatory processes are associated with oxidative stress initiated by production of reactive oxygen species which can function as signaling molecules in many cell types.

Considering the previous studies, it is important to develop new strategies for design of new multifunctional coatings with immunomodulatory capabilities which can permit the reestablishment of ECM support and maintain the physiological needs of the islets. In this respect, the LbL approach offers opportunities for integration of the inherent advantages of both islet microencapsulation and surface modification approaches. For example, multifunctional LbL materials designed from non-toxic biologically-active polymers can provide novel immunoprotective and anti-inflammatory coatings crucial for prolonged islet viability and functions.

5. Acknowledgment

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