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Patented Novelties in Immunoisolation for the Treatment of Endocrine Disorders

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Abstract: Immunoisolation is based on the principle that transplanted tissue is protected for the host immune system by an artificial membrane. During the past decades a number of different approaches of immunoisolation have been described. The approaches include (i) intravascular devices, which are anatomized to the vascular system, (ii) extravascular macrocapsules, which are mostly diffusion chambers transplanted at different sites, and (iii) extravascular microcapsules. Many reviews describing the advantages and pitfalls of the different approaches of immunoisolation have been described during recent years. Almost none of these reviews however describe the technical advances and (pre)clinical results described in the numerous patents on the subject. Therefore this review presents the recent novelties described in patents related to immunoisolation of tissue.

Keywords*:* Microencapsulation, macroencapsulation, islets, diabetes, alginate, immunoisolation, insulin, vascularization, bioartificial, liver, kidney, pancreas.

INTRODUCTION

One of the largest fields of application of immunoisolation is for the treatment of Diabetes. In order to illustrate the background and the potential advantages of immunoisolation for the treatment of endocrine disorder [1], we will first give a background of the general issues associated with treatment of Diabetes.

Administration of insulin for the treatment of insulin-dependent diabetes is still associated with serious complications [2]. Intensified insulin treatment has been shown to delay the onset and to reduce the progression of diabetic complications [3] but it requires multiple daily injections, frequent monitoring, dosage adaptations and, thus, patient compliance [4,5]. Also, it is associated with life-threatening episodes of severe hypoglycemia and with hypoglycemia unawareness.

A major goal in the treatment of insulin dependent diabetes is to improve the patient's quality of life by providing the patient with an insulin source that regulates the glucose levels on a mandatory minute-to-minute basis. This can be achieved with an endogenous insulin source. Basically, there are two options to provide the diabetic patient with an endogenous insulin source. These are transplantation of the whole pancreas and transplantation of only the islets of Langerhans. Transplantation of the whole pancreas is already a well-established mode of treatment with a worldwide experience of more than 15,000 cases [6,7]. Results have substantially improved during the past two decades and presently patient and one-year graft survival rates almost equal to those of routine kidney transplantation (respectively 98% and 85%). A successful pancreas transplant provides almost normal glucose homeostasis, but it requires life-long immunosuppressive medication and is associated with major surgery and high morbidity [8,9]. Since it is still unclear whether the benefits of a pancreas transplant over continued insulin treatment out-weighs, the disadvantages, most transplant centers still restrict themselves to combined pancreas and kidney transplantation in diabetic patients with end-stage renal failure [6,10].

Islet transplantation, in contrast to pancreas transplantation, requires no major surgery. Recent improvements in the technology are the administration of non-glucocorticoid immunosuppression (sirolimus, tacrolimus, daclizumab) by the Edmonton-group which is associated with one-year graft survival of 100% of the transplanted diabetic patients [11]. These advances have led to a tremendous growth in the number of research groups aiming on human islet transplantation. Unfortunately, not all these groups have achieved the same level of success as the Edmonton group which is usually attributed to different degrees in 'experiences' in efficacious isolation of functional islets [12,13]. However, in spite of the inability of many groups to fully reproduce the Edmonton results, the general impression is that recent advances of the Edmonton group have brought islet-transplantation close to wide-spread clinical application.

Another pertinent advantage of islet transplantation over whole pancreas transplantation is that islet transplantation might be achieved without immunosuppression by methodologies such as immunoisolation. Immunoisolation is a technology in which islet-cells are enveloped in semipermeable membranes that are impermeable for the detrimental effect of the host immune system but are permeable for nutrients, glucose, and insulin.

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IMMUNOISOLATION

The concept of immunoisolation is not new. It dates back to 1933, when Bisceglie *et al.* [14] replaced the endogenous pancreas by insulin producing tissue encapsulated in a semipermeable membrane to study the effects of the absence of vascularization on the survival of tissues. At that time Bisceglie *et al.* [14] was mainly interested in vascularization and did not claim the principle applicability of the membranes as a immunoprotective device. The first to recognize this principle was Algire [15] in 1943 who observed that graft failure could be delayed by encapsulating allo- and xenogenic tissues before transplantation. After that period numerous groups designed and applied immunoisolation for the cure of a broad range of diseases such as Hemophilia B [16], anemia [17], dwarfism [18], kidney [19] and liver failure [20], pituitary [21] and central nervous system insufficiencies [22], and diabetes mellitus [23].

Immunoisolating devices have been proposed in all kind of shapes, sizes, and materials. Nowadays, two major designs of encapsulation are being distinguished: intravascular devices and extravascular devices. These two approaches will be discussed in the next section.

INTRAVASCULAR DEVICES

A pertinent advantage of intravascular devices is that the islets in the devices are in close contact with the blood stream. This implies a fast exchange of glucose and insulin and a fast supply of nutrients. The composition of the families of intravascular device is quite similar. It is usually composed of a microporous tube with a relative large lumen. The blood flows through this lumen. These tubes are usually collectively packed in a polymer housing within between the islet cells [24,25]. The device is anastomosed to the blood stream of the host. Many different types of intravascular devices have been tested and found to induce normoglycemia in diabetic rats [26], dogs [27] and monkeys [26]. Notably, however, all devices required systemic and intensive anticoagulation. In spite of this anticoagulation, the fast majority of devices failed due to thrombus formation in the lumen of these small diameters artificial capillaries. As a consequence most devices failed within several weeks after successful transplantation.

A major modification in the devices to prevent thrombosis was the introduction of a larger tubular lumen which in some devices went up to an internal diameter of 5-6 mm. These devices were much more successful than the small bore devices as they showed no significant thrombus formation for periods of seven weeks in the absence of systemic anticoagulant therapy [28]. This success is in part explained by the high flow rates through the device which prevents adhesion of cells to the membranes [29].

The large-lumen intravascular devices were tested in allo- and xenogenic combinations in diabetic dogs [30-35]. In spite of the lack of significant thrombosis a new problem arose. The high flow rates in the device interfered with the efficacy of the implant as an endogenous pancreas. With the new design, two devices per recipient instead of one were required to achieve adequate secretion capacity while maintaining the same numbers of islets per device [36]. Also, it was found that it was not possible to load the device with an islet-tissue density higher than 5-10% of the volume [21], indicating a drawback in exchange of nutrients and oxygen from the arterial blood and the islet-containing chamber.

Although the intravascular devices have some theoretical advantages over other types of devices, the problems mentioned above have not been solved yet. The intravascular design is associated with threats that any type of vascular prosthetic surgery meets. It contains a serious threat for the development of thrombosis, either primary or secondary to intimal hyperplasia at the venous anastomosis, defects of the device, or infection. This is a major drawback for wide-spread application in large numbers of diabetic patients. This is probably why this approach is abandoned for application in diabetics since any alternative to conventional insulin treatment should preferably carry no additional risk [2]. Notably, however, concepts based on these approaches are presently proposed for bioartificial livers and kidneys [37-40]. It therefore might be that the concept will be revisited in the near future [37,40].

EXTRAVASCULAR DEVICES

A major advantage of extravascular devices is that they can be implanted with minimal surgery and are not associated with major risks such as thromboses. As a consequence the devices are subject of investigation in many academical and industrial laboratories. Extravascular devices can be categorized into two different types of devices, i.e. the extravascular macrocapsules and extravascular microcapsules.

The extravascular macrocapsules can be implanted with minimal surgery in different sites such as the peritoneal cavity [41-44], the subcutaneous site [45-51], or the renal capsule [52]. The geometry of macrocapsules may be planar in the form of a flat, circular double layer or tube-like as a so-called hollow fiber [53]. An advantage of the extravascular macrocapsules is that they can be readily retrieved.

The tube geometry is preferred over other geometries by many groups because of its high degree of biocompatibility [54]. Also extravascular devices may provoke inflammatory responses in the recipients but the consequences for the hosts are never as deleterious as with thrombosis in the intravascular devices. Usually, the host-response is deleterious only to the function of the encapsulated tissue and has no or only minimal risk for the recipient. The host responses are usually biocompatibility issues or problems related to toxicity and activation of non-specific foreign body reactions resulting in adhesion of inflammatory cells to the devices and necrosis of the encapsulated tissue [55].

Many have been the efforts to introduce new, better, and most importantly more biocompatible materials to the concept of both macrocapsules and microcapsules. Reviews discussing the academical literature with pro- and contra's of new materials and concepts are numerous. Those described in patents are rarely taken into account and only in some occasions published in academical journals. This has been the main rationally to write this review in which we discuss the concepts described in some recent patents in view of future clinical application.

ONLY A FEW PATENTS ARE APPLICABLE TO CELL-ENCAPSULATION

Going through any of the databanks available on patents will result in numerous patents dedicated to inventions related to encapsulation that mention cell-encapsulation as one of the potential fields of application for their patents. The vast majority of these applications are however meant for processes or technical applications that have no direct connection with cell-encapsulation and cannot, even after significant adaptations be applied as an adequate technology to envelop cells. The reason is that the majority of these technologies apply ingredients or processes that are not compatible with functional survival of cells. One of these fields of application of encapsulation is for electrophoretic media. Numerous patents can be found from the Massachusetts Institute of Technology and the E Ink company [56] but none of them can be realistically applied for encapsulation of cells since it involves toxic components for viable cells. In the present overview, we have mainly concentrated on the recent patents that propose significant modifications to immunoisolating capsules and have not been published in the common scientific literature that has been recently discussed in reviews [55,57].

NOVEL MEMBRANES FOR ENCAPSULATION

Prevention of cellular overgrowth of capsules is considered to be a crucial factor in biocompatibility of microcapsules. For some applications of biomaterials, such as implantation of artificial joints, growth of host cells and coverage of the implant with host-cells is considered a benefit since it promotes the functional performance of the implant [55]. This is different for capsules enveloping cells. The adherence of host cells on the capsule surface is considered to have negative effects because of reduced diffusion of oxygen and nutrients to the encapsulated graft resulting in necrosis of the enveloped cells [58,59]. In addition, the cells on the capsule surface are found to be mainly inflammatory cells secreting cytokines and chemokines that may have a negative effect on graft function [60].

In the past decade many groups have studied the applicability of hydrogels for extravascular encapsulation. Hydrogels provide a number of features which are advan-tageous for the biocompatibility of the membranes. Firstly, as a consequence of the hydrophilic nature of the material, there is almost no interfacial tension with surrounding fluids and tissues which minimizes the protein adsorption and cell adhesion. Furthermore, the soft and pliable features of the gel reduce the mechanical or frictional irritations to surrounding tissue [61,62].

Until the end of the previous century most applications were composed of a one or two layer systems. Most of these systems were having limitation in respect to desired mechanical stability, capsule size, and most importantly surface properties. The part of the capsules that determines the immunological response of a recipient is mainly the surface. The fact that the proposed systems of before 2000 only had very limited possibilities for variation has been the main rational for many academic and industrial groups to propose novel systems with more versatile properties. A pioneering patent in that respect was filed by Wang *et al.* [63]. Wang proposed a multicomponent polymer capsule which allows for specific modification of capsules such as capsule size, wall thickness, mechanical strength, permeability, and surface characteristics. The capsule characteristics can be adjusted and optimized for various, specific applications. Only a limited number of polymer combinations were suitable for the proposed system due to biocompatibility issues. Of the thousands of tested combinations only the combination of sodium alginate (SA), cellulose sulphate (CS), poly(methyl-enecoguanidine)hydrochloride (PMCG), calcium chloride ($CaCl_2$), and sodium chloride (NaCl) were found to allow for building up suitable multipotent capsules. The principle applicability of the methodology was tested with pancreatic islets encapsulated in this multicomponent capsule. Islets in the new capsule reversed diabetes in both chemically-induced and spontaneous diabetic (NOD) mice. Encapsulated rat islets demonstrated glucose-stimulated insulin secretion *in vitro* and reversed diabetes *in vivo* in mice. A major disadvantage of the proposed methodology is that it is very complicated and not available for laboratories with conventional equipment for biomedical research. This is probably the reason that the application of this technology is still limited to inventors of the system.

As indicated above, unfortunately not all hydrogels, have the characteristics that are preferred for cell-encapsulation while some hydrophobic materials have features that are desirable for application in cell encapsulation. For instance, the hydrophobic polytetrafluoethylene (PTFE) is preferred in many applications for its chemical resistance, versatile porosity and its lack of biodegradability. Also PTFE is already generally applied in clinical reconstruction materials and FDA approved. Unfortunately, PTFE is highly hydrophobic and not compatible with survival of encapsulated cellular grafts. One recent invention [64] provides a potential solution for this obstacle in the use of hydrophobic materials such as PTFE in encapsulation of cellular grafts: the inventors propose to apply an external coating on the PTFE in order to increase the hydrophilicity of the surface. This coating is proposed to consist of polyvinyl nucleophilic polymers and an urethane or a blocked isocyanate. Although this methodology is principally proposed for industrial applications such as for water-filtering, the method has highly applicable features for cell-encapsulation. It allows for precise and versatile regulation of the pore size and by varying the degree of cross-linking or type of polyvinyl nucleophilic polymer the surface can be adapted to the specific requirements of the application. This is especially preferable for cell-encapsulation since it has been shown that the requirements of surfaces of capsules vary depending upon the site of implantation. Notably the invention has been described for PTFE but is plausibly applicable to other hydrophobic materials.

An issue that has been an Achilles hill for encapsulation for many years has been the mechanical stability. Many hydrogels are based on electrostatic interactions and therefore considered to be susceptible for degradation. This is specifically a pitfall for areas where long term function is required such as with the artificial liver, kidney, and pancreas. Hubbell *et al.* [65] proposed in a recent patent to solve this potential problem by photopolymerization of water soluble molecules. The authors propose a number of technologies for different applications or requirements. The different methods apply a 'cell-friendly' polymerization method containing water-soluble macro-polymers or molecules that can be further polymerized to

applicable macromolecules. In principle the membranes are stably crosslinked using photoinitiators (eosin) and cell-friendly radiation such as visible or long wavelength UV light. The reactions occur via suspension-polymerization or by interfacial polymerization. An advantage of this system is that it allows for direct building of a membrane on the cells but also for addition of an additional layer around the capsule. This might be beneficial for encapsulation of cell-types with specific requirement such as for example primary beta-cells or hepatocytes that require specific survival factors. Within this application we might create a different inner-capsular milieu that facilitates functional survival while the outside may consist of a biocompatible, strong immunoprotective membrane. The inventors mention in their patent that it is applicable for encapsulation of pancreatic islets in the alginate-poly(l)-lysine system. Unfortunately, the methodology is with conventional laboratory equipment cumbersome to perform. However, with modifications and adaption of the technology as proposed by Pilon *et al.* [66] this might change. Pilon *et al.* [66] proposed in 2006 a novel methodology and device for microencapsulation of liquid substances and ultrasonic atomization. The device produces capsules with a diameter of 0.1 μm tot 1000 μm in diameter. The capsules are formed by a novel device containing a housing through which a laminar flow is created. Capsules are formed by ultrasonic sound and droplets are subsequently crosslinked by an UV beam located in the device. By this novel methodology, photopolymerized shells can be formed in one step in a high throughput fashion.

METHODS TO INCREASE THE INTRA-CAPSULAR BIOCOMPATIBILITY

The interior biocompatibility is usually defined as the (cyto)compatibility between the biomaterial and encapsulated tissue. It is an underestimated factor in the longevity of a graft. Many immunoisolating devices demonstrate excellent biocompatibility when the interaction with the host is considered but it is quite often not compatible with optimal functional survival of the cells, e.g. it has been shown that encapsulation of islet-cells in capsules is associated with loss of up 80% of the graft. This is not acceptable when scarce donor tissue is applied [67-69]. Not surprisingly many groups nowadays not only focus on the exterior biocompatibility but also the intracapsular biocompatibility by introducing new biomaterials or bioactive substances in the capsules in order to prolong the functional survival of the encapsulated cells. In a recent patent, Stewart *et al.* [70], proposed an invention to include tailor-made cocktails of biological substance to promote functional survival of a wide variety of cells. They especially claim integrins as bioactive molecules of choice to induce survival of cells. Integrins such as DCAM, ICAM, and VCAM have been shown to be essential in both viability and function of a wide-variety of cells [71,72]. Integrins do stimulate a series of sequences of intracellular processes that are associated with viability and with the function of the hormone-secreting apparatus of the cells. The authors demonstrate that both viability and function of a variety of cells is increased when integrins are included in either agarose or polyHEMA capsules. To retain the integrins in the capsules the authors propose to include extracellular matrixes in the capsules such as collagen, fibronectin, laminin, or any other integrin-binding protein or synthetic molecule. A critical note however is that all the data presented in the patent are *in vitro* observation and not *in vivo* findings. Many of the mentioned bioactive molecules have a long half-life time *in vitro* but are broken down *in vivo* within several minutes due to specific enzyme-activities [73,74]. Although, promising it should be demonstrated that the functional-survival promoting activity of the integrines and its chaperon will also be observed *in vivo* in order to propose this as an applicable technology.

Another approach was described by Ameer *et al.* [75]. These inventors propose a three-dimensional synthetic novel poly(diolcitrates)-based nanocomposite that is biodegradable and biocompatible. According to the claim the construct provides natural strength and pliability required for cells to grow and function under *in vivo* circumstances. The inventors claim a very broad field of applications varying from medical bandage to reconstruction surgery, tissue engineering and also for immunoisolation by inclusion in microcapsules and macrocapsules. The nanoparticles should give cells a 'natural' environment allowing growth of cells and an optimal functional survival. Although, this might be true, the inventors do not take into account that different cell types have specific requirements. It is highly questionable that the nanocomposites provide the whole array of requirements or have the versatile properties to accommodate a broad range of cells.

A more versatile methodology to accommodate encapsulated cells was recently claimed by Kizilel [76]. In their invention they envelop pancreatic islet-cells with a thin layer of poly-(ethylene glycol) with an incorporated factor that facilitates insulin release, i.e. glucagon-like peptide 1 (GLP-1). The methodology is not meant to immunoprotect the islets but only to increase their functional survival. Similar principles may be applied to other cell-types with specific requirements.

Some researchers propose biological strategies to prolong the survival of encapsulated tissue. Cheung *et al.* [77] state in a recent patent that a deficiency in the current encapsulation technology is that passive material barriers cannot protect biological cells from exposure to cytokines and other small, diffusible cytotoxic molecules produced by activated immune cells. These cytotoxic molecules are according to the inventors responsible for the destruction of cells in the capsules. To solve this issue the inventors applied a system that actively and locally suppresses the immune response using Fas-receptor binding agents. The background of their claim is the following. T-cells are immune cells responsible for the deletion of foreign cells. A characteristic of activated T-cells is that they present Fas-ligand. Normally if Fas is bound to the Fas-ligand, the T-cell will go into apoptosis and disappear. Cheung *et al.* [77] propose to include Fas-like molecules in the system in order to delete T-cells that kill the encapsulated cells. Although this invention is innovative, it will probably have only limited contribution to survival of encapsulated cells. The vast majority of responses against encapsulated tissue are aspecific of nature. T-cells are not involved in the type of responses.

DEVICES FOR CELL-ENCAPSULATION

Many have been the devices described for containing encapsulated cells as extravascular and intravascular device. During the past decade many innovative geometries and designs for both extravascular and intravascular devices have been described [57,78].

Dionne *et al.* of the Rehoboth company [79] described in their patent an extravascular device with a claimed high degree of biocompatibility that allows for implantation of cells for the treatment of a large range of disorders varying from neurological disorders to the treatment of Diabetes. The innovative modification of their invention over other devices is the inclusion of an inner support system in the extravascular device that overcomes the obstacle of undesired bending or even breaking of extravascular devices as the consequence of mechanical friction of the device in the implantation site. This inner support system in the extravascular device consists of a cylinder shaped rod. This rod may be coated with cell-adhesive substances or growth factors to enhance the functional survival of the graft. The rod may be hollow in order to allow influx of for example administered oxygen that support the functional survival of cells. This is beneficial for endocrine cells such as pancreatic islets and hepatocytes that require high, arterial oxygen levels to survive and function. The proposed geometry is as follows. The capsules contain an inner support, a core which contains the cells, surrounded by a peripheral biocompatible but semipermeable capsule. The inner support is usually prepared of other materials than the outer semipermeable membrane. The inner support should be non-toxic for the cells but sufficiently strong to maintain the integrity of the device. Proposed materials are acrylates, urethanes, silicones, PVC, PAN/PVC, and epoxies. Also polymeric or metallic shaped memory materials may be used. For the outer membranes the authors propose a highly biocompatible material. They propose either polyacrylates, polyvinylidenes, polyvinyl chloride, polyurethanes, polystyrenes, polyamides, cellulose acetate, cellulose nitrate, or polysulfones. Notably these are all polymers that are either being used or are being proposed as suitable polymers for medical implants or for bioreactors. The authors of the patent do not mention which polymers have been applied in their prototypes but they demonstrate excellent glucose induced insulin responses from islet aggregates encapsulated in their devices.

Another innovative device was described by Antanavich *et al.* [80] who describes a thin sheet containing fibers that facilitate replication of cells and facilitates diffusion of essential nutrients and oxygen. Surprisingly the inventors do not claim specific materials to produce the device. This is remarkable as these kind of devices should have a specific rigidity in order to prevent breaking and a sufficient pliability to prevent frictional irritation in the implantation site. The claim is more directed to the concept and the inclusion of trophic factors to facilitate prolonged survival of the graft as proposed by many of the patents mentioned in the current review.

The last device that has received minor attention in scientific literature but may have interesting features for future clinical application is an immunoisolating device that allows storage for long duration prior to application. These kind of storage compatible devices are preferred for clinical applications since it would allow banking of devices and immediate application in patients upon request. One such invention that claims such a device is of Fong-Fu Chou [81]. They applied in patients suffering from osteoporosis a small number of 4×10^5 parathyroid cells encapsulated in TheraCyte capsules. The devices were efficacious for prolonged periods of time but failed after several weeks. The claims for application in the patent are broad. Probably, too broad since the limited period of functional survival of cells in these kind of devices qualify the devices for application in diseases that require short-term intervention such as pain relief, regeneration, and acute osteoporosis. They cannot be applied for disease where long-term intervention is required such as for diseases such as Diabetes.

A last but certainly not least innovation was recently described by Dufrane *et al.* [82]. The inventors describe a novel methodology for macroencapsulation of cells in extravascular devices that promote both the intracapsular as well as the extracapsular biocompatibility [82]. The inventors apply a collagen matrix prepared from of the human fascia lata. Notably, this is not only collagen but a mix of bioactive molecules present in the extracellular matrix. The matrix is chemically treated to remove prions and to decrease its immunigenicity. It is applied to accommodate the functional survival of the cells. The cells are seeded on this matrix and subsequently enveloped by a capsule composed of modified alginate. The patent decribes the application of propylene-glycol alginate which is subsequently gelled with aluminium ions. Possibly this alginate layer is also modified with RGD©. This RGD (Novamatrix, FMC Biopolymer, Oslo Norway) is a cell attachment protein of up to 10 aminoacids long. This whole complex is again encapsulated by an alginate layer that is highly biocompatible. It is not mentioned however whether this is again propylene-glycol alginate in the presence or absence of RGD. The inventors reinforced the mechanical stability of the device with a so-called 'clip'. This clip is a mesh that provides mechanical stability and rigidity in the device. When tested in a xenogenic setting with pancreatic islets in chemically induced diabetic primates, it was shown that the devices induced normoglycemia for prolonged periods of time. One graft failed after 16 weeks while three primates remained normoglycemic for the study period of 24 weeks as shown in (Fig. (**1**)). The invention and described results are promising as it has been far from simple up to now to induce normoglycemia with porcine islets and also it has never been shown before that normoglycemia can be achieved for such a long time with a xenograft in primates. Unfortunately, the procedure, including the applied polymers is not described in too much detail which interferes with reproducibility by independent experts.

INNOVATIONS IN INTRAVASCULAR APPROACHES

As outlined in the introduction testing of intravascular devices is not done anymore for application in Diabetics since the risks associated with the devices do not outweigh the reduction in quality of life associated with insulin therapy. This however should not be interpreted as a suggestion that the developed technology is not applicable for other fields of application. Some aspects of the intravascular immunoisolating devices can be of great value for the development of the bioartificial liver or kidney where also some degree of immunoisolation seems to be required. One such invention that was originally proposed for the treatment of diabetes was described by Gore Hybrid Technologies [83]. The inventors describe a novel cell macroencapsulation device with a pliable core that has the advantage that cells can be readily introduced into the device with not more than minimal shear force. These features

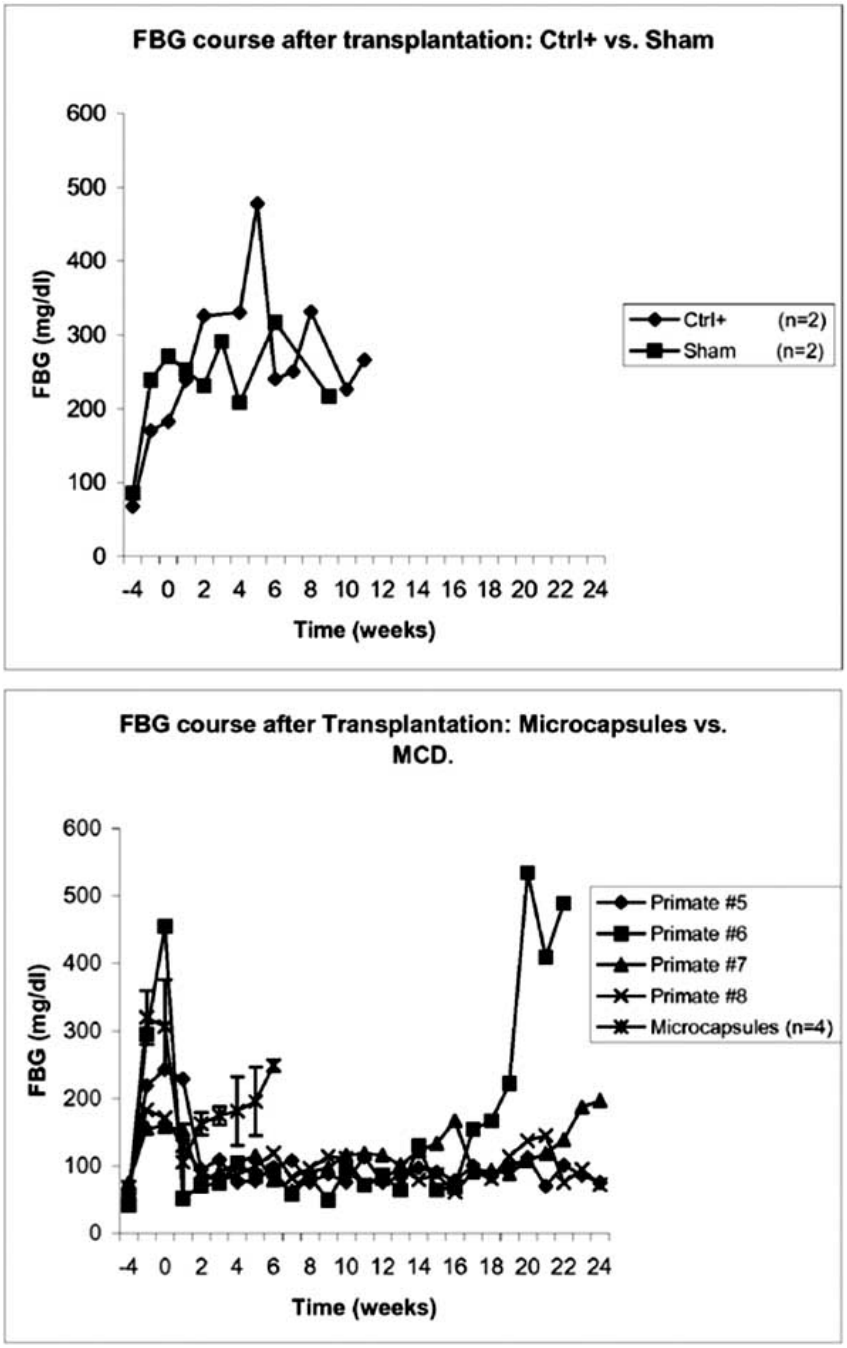

Fig. (1). The fasting blood glucose (FBG) levels after transplantation of the macrocapsules described by Dufrane *et al*. [82]. The upper graph showes the FBG levels of primates receiving a sham surgery with empty capsules or a control graft of nonencapsulated free procine islets. The lower graph shows the results of individual primates receiving a porcine islet graft in the macrocapsules and that of primates receiving microencapsulated islets. Figure obtained from Dufrane *et al*. [82].

are especially beneficial when using primary cells from scare donor materials such as tested for the artificial liver and kidney [37,40]. The device has many adaptations to existing concepts that allow for fast and safe inclusion of cells. This is not only beneficial for the functional survival of the cells but also for minimizing the risk of bacterial contamination of the cells. The device can be applied as an intravascular device either extracorpeal or intracorpeal. The device is also proposes to be a functional bioreactor.

IMMUNOISOLATION AS A TOLERATION TECHNOLOGY

The concept of immunoisolation is enveloping cells in a semipermeable but immunoprotective membrane to circumvent rejection. Although, the concept is usually proposed as a technology to cure disease by replacing disfunctioning or loosed cells, some patents claim new fields of applications. In a recent patent Latta *et al.* [84] present the concept of immunoisolation as a method to treat Diabetes through induction of

immunological tolerance. The inventors claim that the implantation of a specific dose of cells or tissue in an immunoprotective membrane can, by slow release of antigens, induce a state of tolerance in the patient. This would induce after a certain period complete tolerance for a second graft and avoid the application of immunosuppression. The inventors claim that this is not only applicable for allogenic tissues but also for xenogenic tissues which would facilitate the application of organs obtained from animal sources. This would solve one the largest obstacles of organ transplantation of this moment, i.e. donor-shortage. The principle applicability of the methodology was shown in a mouse study in which 100 encapsulated insulinoma aggregates were implanted prior to a 'curing' graft (Fig. (**2**)).

CURRENT & FUTURE DEVELOPMENTS

In this review, we have mainly discussed innovative findings from patents that have received minor or no attention in regular scientific papers and reviews. Many findings in the patents are of significant value for the field. To illustrate this: most academical groups move towards the application of hydrogels in order to improve the biocompatibility of the devices and have abandoned biomaterials with hydrophobic properties. This might be a strategy which may have some drawbacks in the near future as some hydrophobic materials have properties that are beneficial for the field [64]. A number of the patents mentioned in the present review propose technologies to combine preferred properties of hydrophobic and hydrophilic biomaterials by either building hydrophilic structures on hydrophobic constructs or by photocrosslinking hydrogels on top of hydrophobic biomaterials [55]. These approaches are receiving minor attention in the academic community illustrating the lack of attention for patent derived knowledge in the academic community.

Unfortunately, not all patents give a background and overview of critical issues that are compatible with current insights. Surprisingly many background discussions in the patents show large discrepancies in insight between academical reviews. Some critical issues are completely ignored. One such issue is overcoming the effects of the tissue responses associated with implantation surgery. The surgery induced activation of the immune system in the immediate period after implantation is a rather newly discovered reaction with a profound, deleterious effect on encapsulated tissue. This immediate response is not directly related to rejection or autoimmunity and requires more intensive studies in order to find means to interfere with the response. This response is responsible for loss of 60% of the islets in an encapsulated graft [85] even when fully biocompatible capsules are applied. Many academical groups develop novel strategies to overcome this obstacle such as inclusion of anti-inflammatory components in the capsules [86]. None of the studied patents provide a possible solution for this issue nor do the patents mention this obstacle in the application of immunoisolated cells.

Another issue that should receive more attention is the increase in vascular access of immunoisolated cells. Extravascular approaches are preferred because of the minimal risk for the patient. The tissue in the capsules however do dependent for their survival in extravascular approaches on free diffusion of nutrient from surrounding tissues. As a consequence of this, tissues in the devices have a limited survival time [85] and may even show some functional impairment [85]. Many are therefore the efforts to improve the nutrition of the tissue. Some are testing methodologies to induce vascularization around the devices [62]. Although innovative this approach has not shown much degree of success. The reason for this is the fact that every vascularization process is associated with a short but deleterious period of inflammation [87] which results in death of a significant portion of the enveloped cells [87]. Some groups, including ours, therefore concentrate on prevascularized construct as artificial transplantation sites for encapsulated tissue. We have designed and reported solid support systems created from PTFE that provoke a strong angiogenic response after chemical coupling to extracellular matrix components and angiogenic growth factors [88]. These systems allow for optimal nutrition of the encapsulated tissue and have the additional advantage that capsules can be retrieved after cease of function or for any other reasons when replacement is required. The system has been shown to be efficacious in rats but obviously needs many modifications and improvements in order to qualify for human application. Surprisingly these kind of devices have not been subject for patents yet. This was rather unexpected as the constructs are being proposed for free nonencapsulated tissue grafts as well.

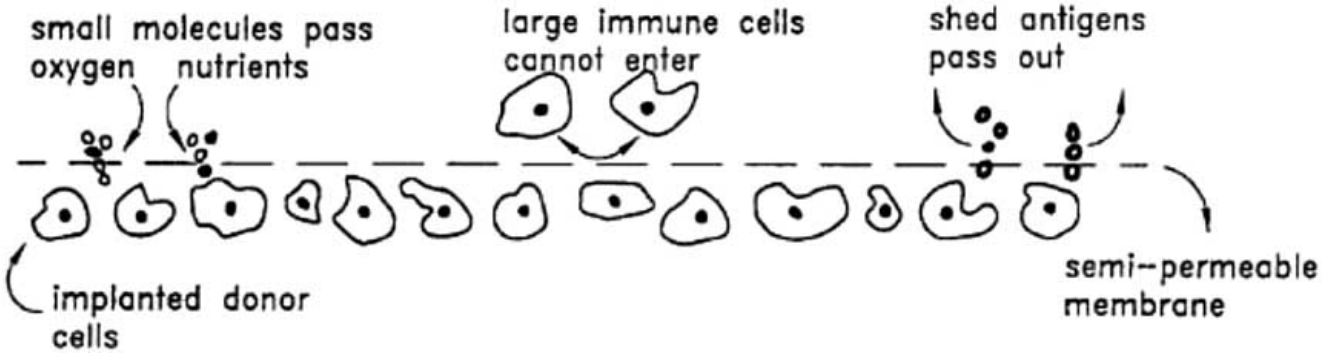

Fig. (2). The principle of 'tolerance' induction by application of immunoisolating devices as described by Latta *et al.* [84]. Shed antigens will diffuse out of the capsules for prolonged periods of time and will induce tolerance for the graft. Figure obtained from Latta *et al.* [84].

In conclusion, patent derived knowledge is valuable and regretfully sometimes ignored by the academical society studying immunoisolation. It contains many modification and procedures that either increases the stability, biocompatibility or the functional survival of the cells. A selection of recent patents that has been ignored in the scientific literature are reviewed in the present manuscript in view of future clinical application.

CONFLICT OF INTEREST

There is no conflict of interest.

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