

Encapsulation of Insulin Producing Cells for Diabetes Treatment Using Alginate and Cellulose Sulphate as Bioencapsulation Polymers

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Abstract

It has long been the holy grail of cell encapsulation to treat diabetes with insulin producing cells that are shielded from rejection or clearance by the host immune system. Indeed, the idea has been around for more than 50 years with the first demonstration in an animal model being published in 1980 and the first patient treated being reported in 1994. In the meantime, more clinical experience has been accrued with the use of encapsulated cells for the treatment of human diabetes. Most of these studies have focused on the use of alginate as the encapsulation material and although some promising data has been obtained and is reviewed in this article, it is still far from being a product that can be routinely applied. Cellulose sulphate has excellent properties making it ideal for cell encapsulation and has been shown to be safe in 27 patients. Moreover, data has been obtained showing safety and efficacy in a rat model of diabetes supporting that cellulose sulphate might be the material of choice for this application.

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Abbreviations: pDADMAC - Polydiallyldimethyl ammonium chloride, Poly-L-Lysine (PLL)

Introduction

Worldwide, in 2013, an estimated 382 million people had diabetes and more than 30 million of these people suffer from type 1 diabetes (also known as insulin dependent diabetes or juvenile diabetes) which results from the autoimmune mediated death or destruction of insulin-producing beta cells that are located in the islets of Langerhans within the pancreas [1,2]. Typically, type 1 diabetes is treated with insulin replacement therapy delivered either via subcutaneous injection or by an insulin pump and it usually requires at least three or more daily insulin injections, which is cumbersome. Insulin administration is accompanied by the need for dietary management and this typically includes carbohydrate tracking as well as careful monitoring of blood glucose levels using glucose meters.

The most common insulins that are currently used for treatment are biosynthetic products produced using genetic recombination techniques [2, 3]. Nevertheless, hypoglycaemic events can occur and these episodes can lead to life threatening incidents as well as long term and ongoing organ damage. The transplantation of beta cells or islets is an alternative treatment that allows insulin to be produced from replacement cells, mimicking the normal physiology much more closely than exogenous insulin injections. This kind of approach leads to a reduction in the incidence of hypoglycemia, improved glycemic control and overall improvement in quality of life [2, 3]. Moreover, beta-cell replacement therapy should also provide great benefit for some people with type 2 diabetes mellitus, where beta-cell insufficiency plays a central role in its pathogenesis [3].

Although initial attempts at islet transplantation showed the potential successes of such a strategy for the treatment of diabetes, there are a number of problems that limit the use of this treatment to relatively few patients due to (i) the need for at least two donor pancreases to treat each time due to poor vascularization and thus hypoxia, patient (ii) graft failure, which occurs within a relatively short period of destruction by autoimmunity and allojection, as well as the toxic effects of immunosuppressive drugs. Moreover, the need for immunosuppression results in vulnerability to infections as well as to various forms of cancer [2, 3].

Cell Encapsulation for the Treatment of Diabetes

Many researchers have turned to immunosuppressive devices as a means to protect transplanted beta cells so that they survive longer in the patient resulting in fewer cells being required. However, it should be noted that, at present, the minimal number of cells required for therapeutic benefit is still controversial. Nevertheless, since these immunoprotective devices are porous, the cells can respond physiologically to high blood sugar by producing insulin but when blood sugar

levels fall then insulin production is down regulated i.e. giving physiological control of insulin production (Figure 1).

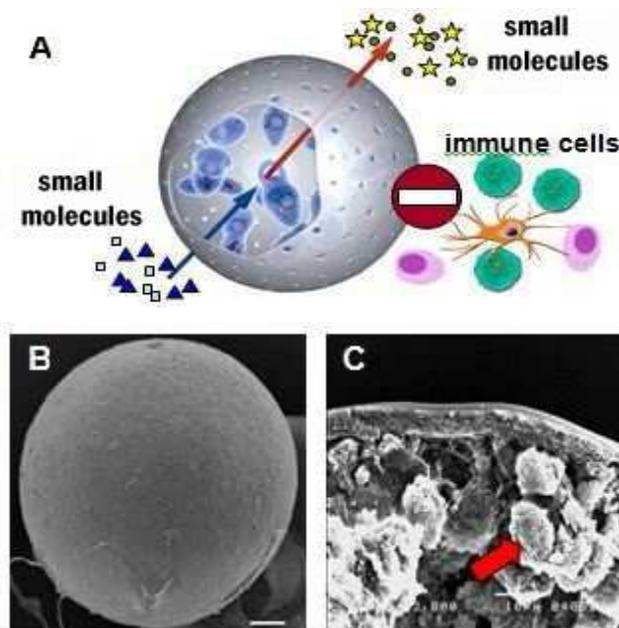


Figure 1: (A) Schematic representation of encapsulated cells and the principle behind the immunoprotection of such cells. Small molecules such as nutrients, blood sugar etc can freely enter the capsule and reach the cells inside so that cells can survive and even divide. Likewise, products produced from the cells such as insulin can be released from the encapsulated cells. Larger molecules and cells of the immune system cannot enter the capsule thus foreign cells cannot be rejected or destroyed (B) Electron microscopic image of an intact cellulose sulphate capsule. (C) Electron microscopic image of a freeze fractured cellulose sulphate capsule. Visible are single cells within the capsule (red arrow). Reference bar in panel B and C represents 100 μm .

Source of cells

In theory a number of different cell sources could be used for encapsulation for the production of insulin including human islets or beta cells as well as those from other species. Most often, porcine islets have been the species of choice even though there are concerns about adventitious agents [4]. Nevertheless, at least most known adventitious agents can be

avoided and cells prescreened [5]. Also as long as the cells remain in the capsule, agents such as viruses might not be released (although there are reports that at least retroviruses can be released from encapsulated cells [6]. Other sources of cells include human stem cells that can be differentiated into insulin producing cells such as mesenchymal stem cells [7] as well as cells that are genetically modified to produce insulin [8]. There has been some concern that certain earlier clinical studies may have been undertaken too soon and the International Xenotransplantation Association has published a consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes that covers preclinical efficacy and complication data required to justify a clinical trial [9].

Encapsulation materials

There are a number of different devices that are being developed to house beta cells or islets for the treatment of diabetes. Lim and Sun first reported in 1980 successful reversal of diabetes in rat recipients of immunoisolated islets for up to 15 weeks [10]. They encapsulated islets in the algae derived polymer, alginate, with Poly-L-Lysine (PLL) and polyethylenimine coatings. Although alginate continues to be a popular choice for cell encapsulation, other materials such as Cellulose Sulphate (CS)(see below), agarose, chitosan, polyhydroxyethylmetacrylate-methyl-methacrylate, copolymers of acrylonitrile and polyethylene glycol are available as well [11]. It is also common to coat alginate with a polycation, which binds to negatively charged alginate molecules.

The most commonly used polycations are PLL as well as poly-L-ornithine, poly-D-lysine and polymethylene-co-guanidine. This procedure has several advantages, such as increasing the mechanical stability of the microcapsule, allowing the control of the membrane porosity and preventing the beads from being dissolved by physiological chelating agents. However, soluble and non-complexed PLL has been shown to be an inflammatory molecule, which leads to fibrotic overgrowth

when it is not properly bound to alginate and it may decrease encapsulated cell viability if used in too high concentrations [12, 13]. Recently improvements have been reported that appear to have addressed some of the issues with the use of alginate [14].

Cells encapsulated in alginate have been used in clinical trials for diabetes. The results from the first type 1 diabetic patient to be treated with alginate encapsulated human islets was reported 20 years ago and showed insulin independence and tight glycaemic control for 9 months after the intraperitoneal injection of the encapsulated capsules islets [15]. However, it should be noted that this patient had previously received a kidney graft and was thus still under immunosuppression so the immunoprotective properties of the encapsulation device was not rigorously tested in this study.

In the last 10 years later, there have been a number of clinical studies with similar outcomes and that demonstrate some degree of safety and bio-invisibility (i.e. absence of a wide array of islet cell-directed as well as anti- MHC class I–II antibodies) but only partial and transient metabolic benefits have been observed in these studies [16-20]. C-peptide could be detected in the first few days after transplantation of the alginate encapsulated cells but standard insulin therapy eventually had to be resumed. A recent clinical study with alginate encapsulated porcine islets cells have shown that although insulin is still required, both the insulin dose and the episodes of unaware hypoglycaemia can be reduced [21].

In some of the trials, there was evidence that the alginate capsules induced a strong foreign body reaction and the capsules were overgrown by fibrous connective tissue (pericapsular fibrotic overgrowth) that may have affected the ability to produce insulin or even the survival of the cells once transplanted [12]. Various factors have been identified as playing a role in the suboptimal biocompatibility of alginate

capsules [22-27] including induction of cytokines that stimulate human monocytes [23] and also the presence of protein contaminants [24].

Although there have been many attempts to improve the biocompatibility of alginate capsules, for example by improving the purity of the starting materials [28] and improving the mechanical strength of alginate capsules, regardless of the type of cell that is encapsulated [14], clearly there are still a number of problems associated with the use of alginate for cell encapsulation [12].

Sodium cellulose sulphate is ideal as a cell encapsulation material [29]. The cellulose sulphate encapsulation technology was originally developed for the treatment of solid tumours such as pancreatic and breast cancer [30-32]. Encapsulated cells were tested in a variety of preclinical animal models as well as in clinical trials and shown to be efficacious. Moreover, the implanted encapsulated cells have a well documented, long term, safety profile in a total of 27 patients - even when they remained in the body for up to two years [30-33].

As we have previously noted [29], capsules consisting of polymers of cellulose sulphate and polydiallyldimethyl ammonium chloride (PDADMAC) (Figure 1) offer a number of advantages as well as unique properties (Table 1). The cellulose sulphate cell encapsulation technology has been used to encapsulate many different cells types, including insulin producing cells such as primary porcine islet cells [34] and hamster beta (HIT-15) cells [35].

Table 1 – some advantages of cellulose sulphate for living cell encapsulation

- Ability to reproducibly source, produce and characterize the starting material.
- Robustness and flexibility of the capsules (permitting delivery through needles or catheters without bursting).

- Good biocompatibility for the cells in the microcapsule.
- Many different cell types and cell lines have been successfully encapsulated e.g. HEK293, CHO cells, beta-cells and pancreatic islets, fibroblasts, epithelial cells, hybridomas and a variety of different stem cells from various sources [38]
- Encapsulated cells are able to grow and survive for extended periods in the capsule but three-dimensional contact inhibition prevents the release of cells from the capsules.
- Good biocompatibility and inertness of the capsules when implanted at various sites in the body.
- Lack of an immune or inflammatory response either to the cellulose sulphate starting solution, the capsule material or to the cells that are protected by it.
- Demonstrated to be safe and well tolerated in three clinical trials and numerous animal studies [30-32, 38].
- Lack of fibrous overgrowth of the implanted capsules.
- Large scale GMP manufacturing of an encapsulated cell medicinal product has been achieved [38].
- Ability to freeze the encapsulated cells for long term storage as well as facilitating shipment around the world.

Both insulin producing cell types showed good viability upon encapsulation and respond to increasing concentrations of glucose by producing insulin in a dose response fashion. Indeed, in the later study, statistical analysis revealed no differences in glucose-dependent cell proliferation, insulin secretion and glucose uptake between non encapsulated and encapsulated HIT-T15 cells and stimulation of HIT-T15 cells with 100 mg/ml glucose resulted in an insulin secretion response that was biphasic [35].

More recently, cellulose sulphate encapsulated porcine islets were tested in immune competent Sprague Dawley rats made diabetic by intraperitoneal injection of streptozotocin. Blood sugar levels were monitored on a weekly basis. Porcine

C-peptide levels and insulin levels were measured using ELISA. Intravenous glucose tolerance testing was performed once a month and revealed that the diabetes was reversed and the rats remained normoglycemic for 4 months after treatment with cellulose sulphate encapsulated porcine islets. After 4 months, the encapsulated cells could be retrieved from the rats and they were shown to be intact. Moreover, immunohistochemical examination revealed that the cells were still viable and producing insulin, demonstrating that long-term in vivo viability is feasible using cellulose sulphate encapsulation and that even xenogeneic cells survive well when encapsulated and implanted into immunocompetent animals [36, 37]. These data appear to be very promising and additional studies are being currently being performed.

Conclusion

In summary, encapsulation of insulin producing cells continues to have great potential for the treatment of diabetes. Moreover, ideally these cells would have to be implanted only once but even if they were to be applied every 3 months, the benefits would greatly outweigh current treatments as well as afford patients that are difficult to treat a treatment option. Although alginate has historically been the method of choice, has most often been used for clinical trials in patients with diabetes and continues to hold much promise, cellulose sulphate encapsulation may offer advantages as well as novel properties as compared to other cell encapsulation methods for the treatment of diabetes (and for a variety of other uses). Moreover, the type of cell that may be encapsulated to treat diabetes may be human or porcine derived beta cells or islets, as well as stem cells that reproducibly can be differentiated into insulin producing cells or even cells that have been genetically modified to produce insulin. All of these cell types can successfully be encapsulated in cellulose sulphate [29].

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