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Direct effect of alginate purification on the survival of islets immobilized in alginate-based microcapsules

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Abstract

Alginate purification has been shown to decrease the host immune response to implanted alginate-based microcapsules, but the direct effect of contaminants on islet cell survival remains unknown. Wistar rat islets were immobilized in calcium alginate beads made with crude vs. purified alginate and then incubated in CMRL culture medium. Islet survival was evaluated at 1, 4, 7, 14 and 27 days post-encapsulation. Islet viability was investigated using a dual staining assay (propidium iodide and orange acridine). The islet cell necrosis and the proportion of apoptotic cells were quantified under optical microscopy and with a TUNEL assay, respectively. Islets immobilized in purified alginate were more viable, and had fewer necrotic centers, a smaller area of central necrosis and a lower number of apoptotic cells. At day 14 and 27 post-encapsulation, respectively, 48% and 23% of islets were viable with purified alginate vs. 18% and 8% with crude alginate ($p < 0.05$). At day 14, the surface area of central necrosis and the number of necrotic islets were more important with the impure alginate (65% vs. 45% and 73% vs. 53%, respectively; $p < 0.05$). We conclude that alginate purification improves the survival of islets that are immobilized in alginate-based microcapsules. These findings indicate that caution should be taken in the interpretation of in vivo experiments, as the results could be explained by either a direct effect on islet survival or a modification of the host reaction, or both. Moreover, it suggests that the effect on islet viability should be assessed during the development of biomaterials for cell encapsulation.

Keywords: Microencapsulation; Islets of Langerhans; Alginate purification; Cell viability

1. Introduction

The transplantation of islets of Langerhans (islets) has improved the blood glucose levels and induced insulin independence in patients with type 1 (insulin-dependent) diabetes [1,2]. However, this treatment has several limitations, including an inadequate reserve of insulin-secreting β-cells [2] and an absence of treatment to prolong islet cell survival, which result in a high percentage of recurrence of insulin dependency (≈90% at 5 years) [3]. In addition, due to the requirement for lifelong immunosuppression, this treatment is usually restricted to relatively advanced cases that have very labile diabetes, whereas improvement of blood glucose control early in the course of the disease would be more likely to prevent long-term complications of diabetes [4].

Microencapsulation of islets in semipermeable membranes has been experimented as a means to avoid immunosuppression by introducing a barrier between the transplanted cells and the host immune system [5–8]. Moreover, Korbutt et al. [9] have shown that immobilizing islets in alginate beads improves islet cell survival in vitro and in vivo. In the latter case, the improvement was not due to immunoprotection, since the experiments were

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conducted in either immuno-incompetent animals (NUDE mice) or a model of syngeneic transplantation. It has been hypothesized that these results are explained by the tridimensional structure of the beads and/or by the development of a favourable microenvironment within the microcapsule. Korbutt et al. [9] have proposed the immobilization of islets in alginate beads as a means to promote islet cell survival during storage. Recently, Qi et al. [10] have shown that human islets that were isolated in Chicago (USA), sent to Trondheim (Norway), immobilized in alginate beads there and sent back to Chicago maintained a similar in vitro and in vivo (when transplanted into immuno-incompetent diabetic mice) viability and function as non-encapsulated islets that remained in the same location (Chicago).

The most widely used system for islet immuno-isolation is the alginate–poly-L-lysine–alginate (APA) microcapsule. In this system, islets are immobilized in negatively charged alginate beads by extrusion of islet-containing droplets into a divalent cation (e.g. calcium or barium) bath, then successively incubated in a positively charged poly-L-lysine (PLL) solution that reinforces the microcapsule strength and allows the control of the membrane molecular weight cut-off, and finally in diluted alginate as an attempt to neutralize the PLL and decrease the microcapsule immunogenicity. Since PLL is known to be immunogenic, a variation of this system is to use non-PLL-coated barium alginate beads.

Although the use of other polymers [11–13] has been tested, none of them have been studied as extensively or have reached the same level of bioperformance as alginate for immobilizing islets. The principal reason is that alginate can be jellified in physiological conditions (room temperature, physiological pH and osmolarity) without producing toxic by-products. Moreover, the rapidity of the gelling process is an important advantage, since it allows the falling alginate droplet to retain the spherical shape that it has acquired in the air. One of the main barriers to the clinical application of this therapeutic approach is the host immune reaction against microcapsules, which leads to pericapsular cell overgrowth. The immune cells around microcapsule release cytokines, which can cross the membrane and damage encapsulated cells. They may also limit the diffusion of nutrients, oxygen, the waste products of metabolism, glucose and insulin.

Much effort has been devoted to decreasing the immunogenicity of microcapsules. As a natural polymer that is extracted from seaweeds, alginate tends to be largely contaminated. In addition, the industrial extraction processes may introduce other contaminants. Methods have been developed for alginate purification [14–17]. The availability of purified alginate has considerably improved the bioperformance of microencapsulated islets in terms of normalizing the blood glucose levels of diabetic animals. Most of these studies have focused on the effect of alginate contaminants and alginate purification on the host response to microcapsules. For example, de Vos et al. [14] have shown that the purification of alginate can reduce the number of overgrown microcapsules 1 year post-implantation in diabetic rats.

Due to its gelling properties described above, alginate is a particularly suitable biomaterial to immobilize islets and form a microcapsule core. In contrast, there is no reason why alginate should be irreplaceable as the polymer that is used for the outer coating of the microcapsules (e.g. poly(methylene-co-guanidine) [18] and polyethylene glycol [19] have both been used). Thus, the most important role of alginate is to form the core of the microcapsule, which is in direct contact with immobilized cells in both systems, i.e. in complete APA microcapsules as well as in non-coated barium alginate beads. In this regard, it is surprising that few data have been published on the direct effect of alginate contaminants on encapsulated cell survival. The objective of the present work was to compare the effect on islet cell survival of immobilizing islets in beads made with crude vs. purified alginate.

2. Materials and methods

2.1. Material and reagents

All reagents and solutions were prepared under endotoxin-free conditions using sterile non-pyrogenic disposable materials and sterile non-pyrogenic glassware. Pharmaceutical-grade sodium alginate Protanal® LF 10/60 (65–75% guluronic acid, $M_w = 135$ kDa) was purchased from FMC Biopolymers (Drammen, Norway) and all other reagents (chloroform, acetone, alcohol, acetic acid, sodium citrate), which were of analytical grade, were purchased from Fisher Scientific Ltd. (Pittsburgh, PA, USA), and were used without further purification. Endotoxins on non-disposable materials were removed by washing for 60 min in Extran soap, then in distilled water, HCl 2 N and sterile water, followed by gas treatment. All manipulations were performed under sterile conditions whenever possible.

2.2. Alginate purification

For the purpose of this study, the Protanal® LF 10/60 alginate was purified using a protocol elaborated originally by Klöck et al. [15] with slight modifications. Briefly, 9 g of alginate powder was incubated in 400 ml of chloroform for 30 min and filtered under vacuum on Whatman No. 4 filter paper. This chloroform extraction was performed three times, then the alginate was dissolved in distilled water to make a 1.5% solution. Next, an equivalent alginate-weight of acid-washed activated charcoal was added to the solution and the mixture was stirred for 4 h. This procedure was repeated once using neutral charcoal. The solution was filtered on 0.22 μm filters, then alginate beads were produced using a 50 mM $BaCl_2$ solution as the jellifying agent. Beads were washed with sterile water and incubated three times

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