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Acta Biomaterialia 2 (2006) 221–227
Acta BIOMATERIALIA
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Biocompatibility and function of microencapsulated pancreatic islets

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Received 29 July 2005; received in revised form 24 October 2005; accepted 1 December 2005

Abstract

Encapsulation of pancreatic islets in alginate is used to protect against xenogenic rejection in different animal models. In this study, several factors, including differences in alginate composition, the presence or absence of xenogenic islet tissue and a transient immunosuppression, were investigated in a model of bovine islet transplantation in rats. A pure alginate with predominantly guluronic acid (Manugel) and an ultrapure low viscosity guluronic acid alginate (UP-LVG) were used. When microcapsules of Manugel or UP-LVG containing 16,000 bovine islet equivalents were transplanted in diabetic rats, we observed normoglycemia for 8.3 ± 0.7 (range 6–12 days) and 7.5 ± 0.2 days (range 7–8 days) on average, respectively. To ameliorate immunoprotection of alginate microcapsules we repeated the same experiments using transient immunosuppressive therapy. Low doses of cyclosporin A (CyA) administered for 18 days after implantation increased the time in normoglycemia, which averaged 27 ± 3 days (range 8–55 days) in Manugel capsules while in UP-LVG capsules it averaged 18 ± 8 days (range 3–39 days). The surface of recovered capsules showed less capsules free of overgrowth in Manugel with respect to UP-LVG alginate. These data were comparable with those observed in empty microcapsules similarly implanted, indicating that the capsular overgrowth was not promoted by the presence of xenogenic islet tissue. In recovered Manugel capsules the percentage of capsules without fibrotic overgrowth was higher than that observed without CyA. The same observation was made in empty capsules. These observations indicate that a combination of a highly purified alginate and short-term immunosuppression prolong islet function in a model of xenotransplantation.

Keywords: Pancreatic islets; Alginate; Microcapsules; Cell overgrowth; Immunosuppression

1. Introduction

Xenogenic pancreatic islet transplantation represents a potential therapy for treatment of insulin-dependent diabetes mellitus. However, this technique requires the administration of immunosuppressive drugs, which are well known to be associated with serious side effects. Xenogenic tissue may be used as alternative resources of organs for transplantation. However, xenotransplantation may also induce stronger rejection, as compared with allogenic transplantation. To control this rejection, several strategies are being developed. One of these is to encapsulate the islets by a semipermeable membrane to separate engrafted cells from host cells [1,2]. In most studies, alginate encapsulation has been used to immunoisolate pancreatic islet in various animal models without immunosuppression [3,4]. With immunoprotection by encapsulation, islets are enclosed in a matrix, which allows for the passage of small molecules like insulin and glucose, but not for the entry of much larger cells and antibodies of the immune system. The most commonly used microcapsules are composed of alginate–poly-L-lysine–alginate. Cells are enclosed in an alginate core which is covered by poly-L-lysine, a polyamino acid that gives the microcapsules semipermeable properties. A second layer that consists of alginate is applied for coverage of the unbound poly-L-lysine group [5]. Allotransplantation of microencapsulated islets in experimental animals has been

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doi:10.1016/j.actbio.2005.12.002

extensively studied [6–8] while not so many studies have been published on discordant xenotransplantation [3,9]. The implantation of encapsulated islets reversed diabetes in xenotransplantation models, but the continuing function of the implants usually required immunosuppression and often generated fibrotic reactions. Despite promptly inducing normoglycemia, microencapsulated islets lose their function within weeks of implantation [10,11] and research is now ongoing with the aim of identifying the mechanisms responsible for failure of graft function [1]. Among other factors, it has been reported that limited graft survival has generally been associated with peri-microcapsular cell overgrowth leading to a thick fibrosis around the microcapsules [12,13]. This cellular and matrix deposition around the capsules may result in a diminished diffusion of oxygen and nutrients to the pancreatic islets contained in microcapsules [13,14] and consequently, in diminished islet function and viability with time. The cause of the cellular overgrowth may be a reaction against the material (the alginate gel) or a reaction against islets embedded within the capsules or some of their components.

We performed the present study to investigate whether microencapsulation alone is sufficient to protect a xenotransplantation of islets from rejection. As a control, we implanted free islets into the peritoneal cavity. We used bovine pancreas as the animal source of islets for our study. In a previous work we showed that calf pancreas is a good and convenient source of tissue for massive islet isolation for experimental studies on xenotransplantation [15]. We decided to use for these experiments alginate microcapsules without poly-L-lysine since usually this layer is covered again with alginate that is directly in contact with the external environment. For this reason we first investigated the recipient reaction using Manugel, a pure alginate with predominantly guluronic acid or an ultrapure low viscosity guluronic acid alginate (UP-LVG) alone. We also investigated whether administration of cyclosporin for a short time after implantation could help in limiting cell overgrowth around alginate microcapsules. We analyzed whether capsular overgrowth is promoted by the presence of xenogenic islets within the capsules or simply depends on alginate material itself.

2. Materials and methods

2.1. Islet preparation

Islets were isolated from pancreas obtained from 6-month-old calves [15] using a modification of the automated method [16] and purified by centrifugation on discontinuous density gradient. Briefly, the main pancreatic duct was cannulated and perfused with collagenase solution (2.7 U/ml, type P, Roche Diagnostic, Mannheim, Germany). After ductal distension the splenic lobe of the pancreas was placed into a perfusion chamber. Collagenase solution was recirculated using a closed loop circuit to obtain pancreas dissociation. When islets appeared free from exocrine tissue in the sample, the dissociation chamber was flushed with 8–10 l of cold (4 °C) Hanks' balanced salt solution (Gibco InVitrogen Corporation, Paisley, Scotland). The digested tissue was collected and purified by centrifugation on Histopaque (1.077 g/ml, Sigma). Islets were collected and cultured at 37 °C in a humidified atmosphere with 5% CO_2 in M199 medium, supplemented with 10% bovine serum, 25 mM Hepes and antibiotics (penicillin 100 U/ml, streptomycin 100 μg/ml, gentamicin 50 μg/ml and amphotericin B 1 μg/ml) (Gibco). We determined islet yield of the isolation by counting the total number of islets equivalents (IEQ 150-μm-diameter islets) using an inverted microscope equipped with a calibrated grid.

2.2. Islet encapsulation

After overnight culture, 16,000 IEQ were suspended in a solution of 1.7% sodium alginate (Manugel DMB, Monsanto plc, Surrey, United Kingdom) or of 2.5% ultrapure alginate (Pronova UP-LVG, FMC BioPolymer, Philadelphia, PA, USA) at a concentration of 3 IEQ/μl. Manugel is a high viscosity, pure sodium alginate. Microbiological tests showed bacteria <5000 cfu/g and yeast and mould <300 cfu/g. We have no data on endotoxin content. UP-LVG is an ultrapure sodium alginate with bacteria viable count <100 cfu/g, yeast and mould <100 cfu/g and endotoxins <220 EU/g. The molecular weight cut-off of the two alginates ranged between 450 and 560 kDa, respectively, for UP-LVG and Manugel, as measured by diffusion of FITC-Ficoll (TdB Consultancy AB, Uppsala, Sweden) of graded sizes using high performance liquid chromatography (HPLC) separation (Chem Station LC-Agilent, Palo Alto, CA, USA). Diffusion experiments carried out with alginate microcapsules at room temperature (A. Remuzzi, personal observation) showed that in 7 h molecules smaller that 20–30 Å of radius freely diffuse in both alginate matrices, while molecules of 110 and 120 Å molecular radius diffuse less than 1% during the same observation time in UP-LVG and Manugel, respectively. Ficoll molecules of these sizes have molecular weights corresponding to those indicated [17].

The islet–alginate mixture was extruded through an air jet droplet generator into a solution of 100 mM $CaCl_2$ solution. The resulting gel beads had diameters ranging from 800 to 950 μm. After complete gelification, the beads were washed in calcium-free Krebs–Ringer, then in Krebs–Ringer–Hepes 25 mM, and then cultured overnight in complete medium at 37 °C.

2.3. Experimental design

We used Munich Wistar Frömter (MWF) rats from our colony [18], originally selected from the Wistar rat, for this study. Animal care and treatment were conducted in accordance with institutional guidelines that are in compliance with national (no. 116, suppl. 40, 18 febbraio 1992, Circolare no. 8, 14 luglio 1994) and international laws and policies (EEC Council Directive 86/609, OJL358-1, December

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