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Preparation and characterization of permselective, biocompatible membranes for the macroencapsulation of pancreatic islets

L. LELLI, N. BARBANI, A. BONARETTI, P. GIUSTI
Dipartimento di Ingegneria Chimica, Chimica Industriale e Scienza dei Materiali, Via Diotisalvi 2, 56126 Pisa, Italy

P. MARCHETTI, R. GIANNARELLI, R. NAVALESI
Cattedra di Malattie del Metabolismo, Istituto di Clinica Medica II, via Roma 55/67, 56100 Pisa, Italy

P. VIACAVA
Istituto di Anatomia e Istologia Patologica, via Roma 55/57, 56100 Pisa, Italy

Polymeric membranes were prepared by mixing poly(vinyl alcohol) (PVA) and poly(acrilic acid) (PAA) solutions (10/90 weight ratio), freeze-drying and crosslinking under vacuum. The membranes were then coated with alginic acid sodium salt, and gelation was accomplished by calcium chloride. The resulting membrane structure was essentially trabecular, with pore size ranging from 50 to 100 μm. Glucose and insulin diffusions reached a plateau at 2 and 32 min, respectively. Immunoglobulins and red blood cells did not cross the membranes. Gel-permeation HPLC showed that the membrane wall was impermeable to compounds with a molecular weight higher than 100 000 D. Biocompatibility of the membranes was demonstrated by showing minimal fibrotic reaction at 3 weeks after intraperitoneal implantation into mice. Porcine islets were placed into the membranes and cultured at 37 °C for 7 days, at which time the incapsulated islets were shown to release insulin in response to glucose. These results support the use of PVA/PAA membranes for bioartificial pancreas studies.

1. Introduction

In the past few years several cases of insulin-independence have been reported following pancreatic islet transplantation into Type 1 diabetic patients [1]. A major problem still limiting wider and more successful application of islet transplantation in humans is the lack of appropriate means to prevent graft rejection. A possible method to circumvent rejection could be immuno-isolation of islets in biocompatible membranes. By this technique, the encapsulated cells are separated from the diabetic host by an artificial membrane which is permeable to glucose, insulin and other nutrients, but not to immunologically active cells and cytotoxic antibodies. At the present time, it is possible either to encapsulate each single islet (microencapsulation), or encapsulate several islets within each single membrane (macroencapsulation). A major advantage with macroencapsulation is the possibility to retrieve the transplanted tissue for successive histological and functional studies. Although some reports have been published showing reversal of diabetes in rodents following transplantation of macroencapsulated islets [2, 3], none of the currently available membranes is completely satisfactory. We therefore considered it of interest to initiate a research programme in order to develop biocompatible, permselective membranes to immunoisolate isolated pancreatic islets. Herein we deal with the preparation, structural characterization, diffusion properties and biocompatibility of original polymeric membranes.

2. Experimental procedures

2.1. Preparation of the membranes

Poly(vinyl alcohol) (PVA, Aldrich Chemie, Steinheim, Germany), with an average molecular weight of 114 000, a syndiotacticity degree of 62% and a hydrolysis degree of 100% and poly(acrylic acid) (PAA, Aldrich Chemie), with an average molecular weight of 250 000, were used for the preparation of the membranes which were then coated by alginic acid sodium salt (AA–Na, Fluka Chemie AG, Switzerland, N° 71238). PAA and PVA aqueous solutions were prepared by adding the solid polymers to distilled water at 70 °C, under stirring. Both the resultant solutions were of 2.5% (w/v) concentration. A PAA/PVA mixture, with a polymer ratio of 10/90 (w/w), was prepared. This mixture was dispensed in samples of 2 ml in Petri dishes. Membranes were then obtained by freeze-drying (− 18 °C for 15 h) and crosslinked by thermal treatment under vacuum at 130 °C for 6 h [4]. Then the membranes were placed into the AA–Na solution

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(1% w/v in saline) for 15 h. Gelation was accomplished by $CaCl_2$ (2% w/v in saline) as previously reported [5].

The morphology of the membranes was evaluated by scanning electron microscopy (SEM). The material was dehydrated through an increasing series of ethanol solutions, critical point dried against CO_2, sputter-coated with gold and observed by SEM.

2.2. Permselectivity studies

The membranes were loaded with D-Glucose (3 ml of a 50% solution), purified A14 monoiodoinsulin (157.455 ± 1.323 cpm, in 3 ml of saline solution), immunoglobulines of the IgG class (anti-insulin antibodies, prepared as described in [6]) and red blood cells. For the glucose diffusion studies (five experiments), samples of the diffusate were analysed at 2 min intervals for 10 min, and glucose concentration was measured by a Beckman glucose analyser (Beckman, Galway, Ireland). In the insulin diffusion experiments ($n = 5$), the diffusates were sampled at 0, 5, 10, 15, 20, 30, 45 and 60 min, and radioactivity was counted by an Auto Gamma 500 C counting system (Packard, Downers Grove, IL). The presence of anti-insulin antibodies in the diffusate was evaluated in three experiments after 30 and 60 min, by incubating samples of the medium with labelled insulin, as previously described [6]. For visualization of red blood cells, the diffusate was centrifuged at the end of a 60 min incubation, and then analysed by light microscopy.

To determine the cut-off of the membranes, we performed experiments by loading the membranes with compounds of varying molecular weights: bovine-albumin (MW 66 000 D), β-galactosidase (MW 116 000 D), myosin (MW 205 000 D). Samples from the diffusate were taken after 3 h of incubation. Both the starting mixture and the diffusate samples were analysed by gel-permeation high-performance liquid chromatography (GP–HPLC) using 0.5 M dipotassium hydrogen phosphate, 0.1 M sodium chloride, at pH 2.5, as eluent.

2.3. Biocompatibility studies

Under general anaesthesia, the membranes were placed into the peritoneal cavity of two balb/c mice, retrieved after 7 and 21 days, and analysed by light and electron microscopy.

2.4. Islets encapsulation

Porcine islets were prepared and cultured as previously described [7]. After 15 days of isolation, approximately 50 islets were loaded into the membranes, which were then coated with the alginate gel. After 7 days the encapsulated islets were challenged sequentially with 60, 300 and 60 mg/dl glucose, in Krebs-Ringer bicarbonate solution, and insulin release was measured at the end of each incubation period.

3. Results

The structure of the prepared membranes was essentially trabecular with pore diameters approximately 50–100 μm. An external layer of alginate was observed to cover the surface (Fig. 1).

Fig. 2 shows the patterns of glucose (a) and insulin (b) diffusion across the membranes as a function of time. Glucose diffusion reached a plateau at 2 min, at which time all the dextrose could be found in the diffusate (97 ± 2%). Insulin diffusion plateaued at approximately 45 min, at which time 32 ± 8% of the

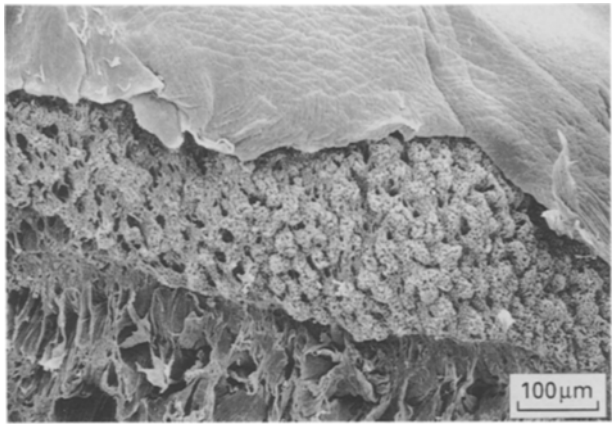
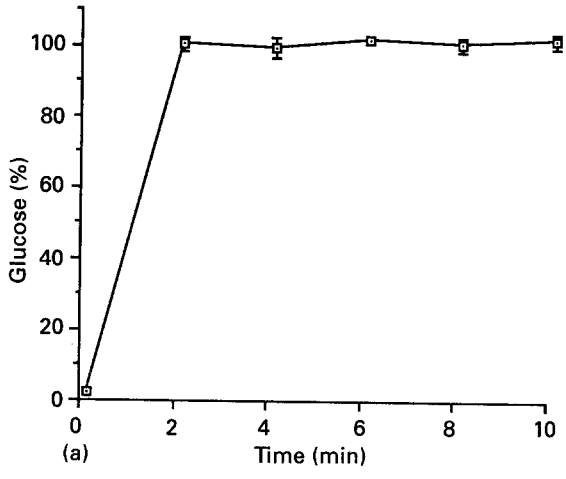


Figure 1 SEM image illustrating the trabecular structure of a section of PAA/PVA membrane and the external layer of alginate.



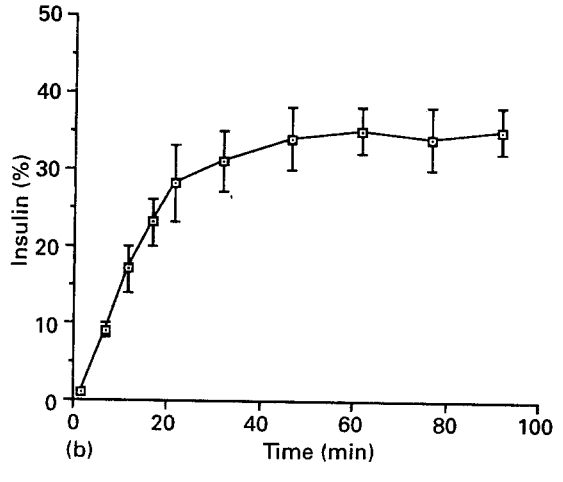


Figure 2 Diffusion patterns of glucose (a) and insulin (b) across the PAA/PVA/alginate membranes as a function of time. The plot averages data obtained from five diffusion experiments.

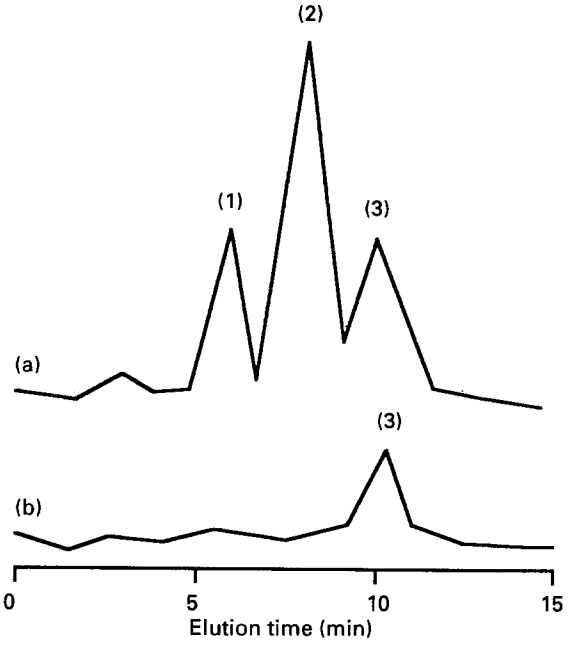


Figure 3 (a) GP–HPLC profile of a mixture of (1) myosin, (2) β-galactosidase, (3) bovine-albumin; (b) GP–HPLC profile of the solutes that diffused across the PAA/PVA/alginate membrane.

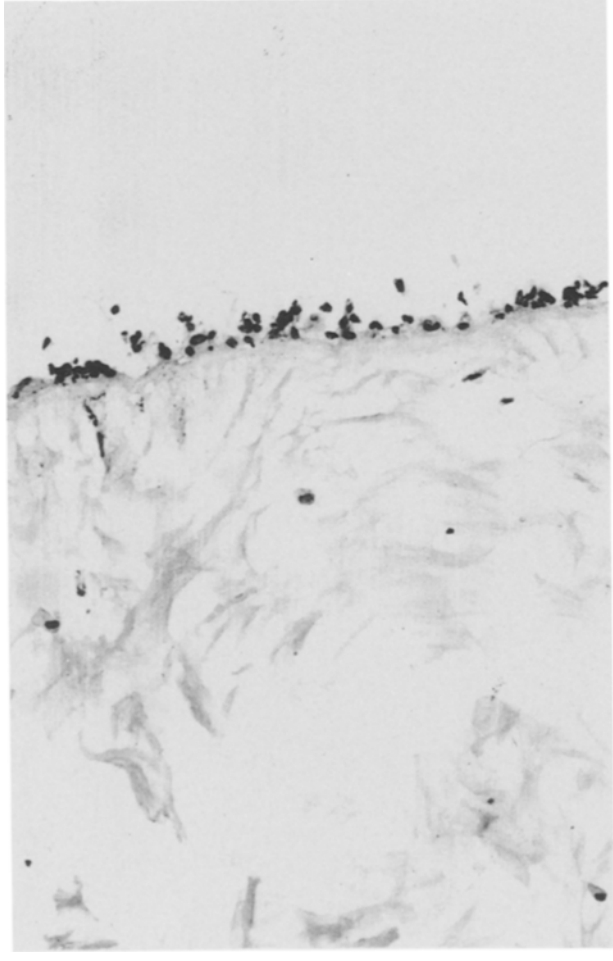


Figure 4 Hematoxilin-eosin stain of PAA/PVA/alginate membrane 21 days after implantation into the peritoneal cavity of balb/c mice (original magnification: × 400).

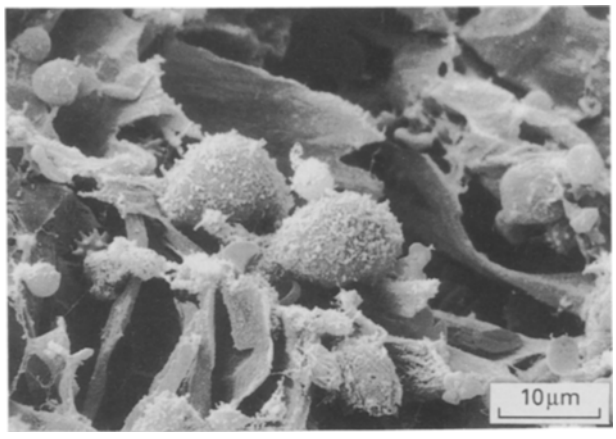


Figure 5 SEM image illustrating the preserved trabecular structure of the PAA/PVA/alginate membrane 21 days after implantation into the peritoneal cavity of balb/c mice.

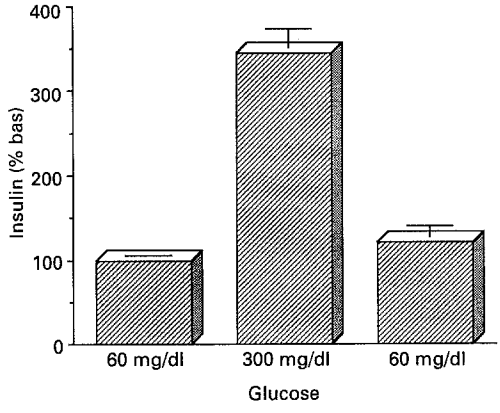


Figure 6 Insulin released from encapsulated islet in presence of 60, 300, 60 mg/dl of glucose after 60 min incubation. Insulin is expressed as a percentage of the basal level. Columns average data obtained from five releasing experiments for each glucose concentration.

loaded dose was released. Immunoglobulins and red blood cells did not cross the membranes.

The results of the GP–HPLC experiments showed that the wall of the membranes was not permeable to compounds with a molecular weight higher than 100 000 D (β-galactosidase and myosin in our experimental conditions) (Fig. 3).

After 7 and 21 days from implantation, the membranes were removed from the peritoneal cavity of the mice and analysed by light microscope. Around the membranes only a minimal inflammatory reaction could be seen by haematoxilin-eosin staining (Fig. 4). The cellular infiltrate consisted of a few lymphocytes, plasmacells and neutrophyle granulocytes, and occasional fibroblasts. Scanning electron microscopy revealed a well-preserved trabecular structure (Fig. 5).

Fig. 6 shows the insulin release from the encapsulated islets after stimulation with glucose: the increase of glucose concentration from 60 to 300 mg/dl caused higher production of insulin, and returning to the basal glucose levels determined a decline of hormone secretion (Fig. 6).

4. Conclusions

Immunoisolation of pancreatic islets by biocompatible, permselective membranes could possibly solve the problem of immune rejection [2, 3]. In the present

study, we demonstrate that membranes prepared with poly(vinyl alcohol) and poly(acrylic acid), coated with calcium alginate fulfilled the preliminary requirements needed to consider these membranes for potential use in the preparation of a biohybrid pancreas. In particular, the membranes were biocompatible, as demonstrated by the presence of a very low inflammatory reaction after 21 day implantation in the peritoneal cavity of balb/c mice, and showed suitable permselective properties as demonstrated by diffusion and GP-HPLC studies. Interestingly, macroencapsulated porcine islets released insulin proportionally to the concentration of glucose in the incubation medium.

Take together, the results of the present study support the use of the membranes we have developed for immunoisolated islet transplantation studies.

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