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# Polyurethane-polydimethylsiloxane (PU-PDMS) tubular membranes for pancreatic islet transplantation. Permselectivity and diffusion studies

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Transplantation of immuno-protected xenogenic islets of Langerhans by a permselective synthetic membrane represents an intriguing possibility to treat the diabetic disease. In this study, polymeric tubular membrane with a porous wall structure and an internal permselective layer (skin) were fabricated by a combination of spraying and phase-inversion of a thermodynamically unstable solution of a biocompatible PU(90%)-PDMS (10%) blend (Cardiothane™ 51) over a sliding and rotating mandrel. Segments of tubular membranes were capped at both extremities according to the "macroencapsulation" technique.

Reference compounds of different molecular masses were used to evaluate capsules permselectivity characteristics, while glucose, insulin and porcine islets of Langerhans were used to study the diffusion properties. The results of these studies showed the capsules to be impermeable to compounds with a molecular mass higher than approximately 40 000 Da, but permeable to glucose and insulin. Pancreatic islets entrapped within the capsules released, over a 2 h period, insulin proportionally to the glucose concentration in the medium without a significant difference in comparison with the amount of insulin released by non-encapsulated islets. Finally, potential drawbacks deriving from an inaccurate capsules sealing and from the severity of the host tissue reaction versus the structural material are discussed.

## 1. Introduction

Pancreatic islet transplantation in humans is limited by the relatively low availability of human islets [1] and the problem of immuno rejection [2]. A possible solution might be to transplant xenogenic islets surrounded by a permselective synthetic membrane which isolates the transplant from its environment [3]. In that case, the membrane material allows the placement of living tissues in an immuno-protected recipient without the need for immunosuppression therapy. The purpose of this functional unit, which is called "bioartificial pancreas", is humoral interaction between the implant and the host recipient. Theoretically, the membrane surrounding the islets must be permeable to nutrients, glucose and insulin, so that the encapsulated tissue may live and function indefinitely in a sequestered environment. At the same time, the membrane must act as a permselective barrier preventing the entry of antibodies and lytic factors of the complement, as well as the inward migration of white blood cells (Fig. 1). Numerous permselective membranes such as the alginate–polylisine–alginate membrane [4], polyvinyl chloride acrylic copolymer [5] and polycarbonate tubes [6] have been used with some success for islet allograft and xenografts in rodents, but any membrane is completely satisfactory. The dominant issue remains the inadequate membrane material biocompatibility, that is the ability of the polymer to elicit a minimal, rapidly stabilized inflammatory reaction in the host's tissues, resulting in the permanent acceptance of the implant without altering its function. An alternative might be that represented by polyurethane materials, which have shown good blood and tissue compatibility in a variety of applications, such as vascular prostheses, blood filters, catheters, pacemaker lead insulators, heart valves, and artificial organs [7–13]. However, notwithstanding these good material properties, to date no scientific papers report using polyurethane materials for the development of a bioartificial pancreas.

In this study bioartificial pancreas units were prepared according to the "macroencapsulation" tech-

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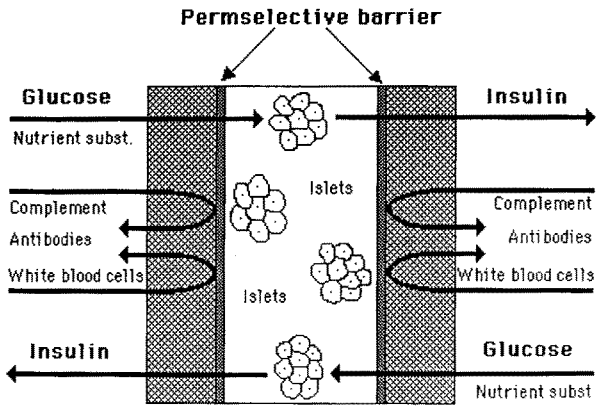


*Figure 1* Schematic representation of a bioartificial pancreas functional unit

nique, which relies on preformed tubular membranes, typically 0.5 to 1.5 mm in diameter, 1 to 3 cm in length, and capped at both extremities. The membrane wall structure is usually spongy with intercommunicating voids and open windows on the external side whereas the inner surface is a smooth, tight condensed polymer layer which controls the permselectivity. The tubular membranes were fabricated according to a spraying, phase-inversion process, previously described to produce porous vascular prostheses [14], using as a membrane material a PU(90%)-PDMS(10%) blend (Cardiothane™ 51), which has shown minimal inflammatory reaction in rat abdominal aorta implantation experiments [14].

To explore both the potential of the fabrication process and of the PU-PDMS material as possible tools to produce bioartificial pancreas units, the tubular membranes' permselectivity characteristics were evaluated. In addition, glucose, insulin and porcine islets of Langerhans were separately entrapped within the membranes, and the diffusion patterns of glucose and insulin were studied.

## 2. Materials and Methods

### 2.1. Tubular membranes fabrication

A commercially available 16% w/v solution of Cardiothane™ 51 (Kontron Instruments, Inc., Everett, MA, USA) [15–17] in 2:1 THF-1,4 Dioxane was diluted, with a highly purified 2:1 THF-1,4 Dioxane solvent mixture (Kontron Instruments, Inc.) to reach a polymer concentration of 1% w/v.

Tubular membranes, 1.5 mm i.d., with a dense inner polymer layer (skin) and an outer porous wall were fabricated as follows: briefly, the skin was produced by first spraying 0.3 ml of the 1% polymer solution over a rotating mandrel. After this step the deposited polymer was dried for 2 min at room temperature. The porous wall was subsequently formed by simultaneously spraying distilled water and a thermodynamically unstable PU-PDMS solution (obtained by adding 16.5 ml of distilled water to 100 ml of the 1% polymer solution). Membrane wall thickness was closely related to the volume of polymer solution sprayed. After 21 ml were sprayed, the process was stopped and the Teflon mandrel with the deposited material was submerged overnight in a bath of deionized water. Then the tubular membrane was removed by reducing the Teflon sleeve diameter with an axial stretching. Finally, it was constantly maintained in distilled water at room temperature until characterized. Further information concerning the processing conditions used to fabricate these membranes are reported in the above cited publication.

### 2.2. Scanning electron microscopy (SEM)

SEM was performed to obtain information on the pore size, shape, and surface characteristics of the luminal side, outside, and cross-section of the membrane. The material was examined by using an AM-Ray 1000A scanning electron microscope. Because the vacuum drying procedures necessary for conventional SEM significantly distort the material by collapsing its sponge-like structure, samples were frozen and then freeze-dried (Labconco) before being spatter coated with gold-platinum (SPI sputter).

### 2.3. Permselectivity studies

Segments of tubular membranes, 20 mm long, were first capped at one end with a drop of a 20% w/v glue solution; afterwards the glue cap was coagulated for 3 min in distilled water. The glue was prepared by dissolving a determined amount of vacuum dried Cardiothane™ 51 in dimethylsulphoxide (a non-cytotoxic solvent). Three membrane segments were filled by inserting in the open extremity a 1.5 mm o.d. flat needle attached to a plastic syringe containing aliquots of a mixture of the reference compounds of different molecular masses lilsted in Table I. The open extremity was then sealed with the same technique described above and the membranes were separately placed for 30 min in plastic cuvettes containing 1 ml of Hank's solution. At the end of the incubation period the medium was analysed by gel-permeation high-performance liquid chromatography (GP-HPLC) with a TSKG 3000 SW column (LKB, Broma, Sweden) on a Waters HPLC system (Waters Associates, Milford, MA, USA), and using as eluent 0.5 M dipotassium hydrogen phosphate, 0.1 M sodium chloride, at pH 2.5

In an additional experiment, three membrane segments were filled with Hank's solution, and were then separately placed in 1 ml of Hank's solution containing immunoglobulines IgG or white blood cells. After

TABLE I Reference compounds used for permselectivity studies

| Compound | Molecular mass |
|---|---|
| 1 Thyroglobulin | 677 000 |
| 2 IgG | 158 000 |
| 3 Bovine-Albumin | 68 000 |
| 4 Ovalbumin | 44 000 |
| 5 Myoglobin | 17 000 |
| 6 Insulin | 6000 |
| 7 Vit B12 | 1300 |

a 30 min incubation period, the content of the membranes was analysed with a Zeiss PMQII spectrophotometer (Carl Zeiss, Oberkochen) and by light microscopy for IgG detection and leucocyte visualization respectively.

## 2.4. Diffusion studies

### *2.4.1. D-glucose*

Four membrane segments were filled with 1 mg D-glucose (corresponding to 50 µl of a 2 g/100 ml glucose solution) as described above, and were then separately incubated in 1 ml of Hank's solution for 20 min. Aliquots of the medium were drawn every 2 min and glucose concentration was measured by a Beckman glucose analyser (Beckman, Galway, Ireland).

### *2.4.2. Insulin*

Three membrane segments were filled with 50 µl of a solution containing HPLC purified A14 monoiodoinsulin [18] as described above. The radioactivity content was 78.200 ± 2.200 c.p.m. The chambers were separately incubated in 1 ml of Hank's solution, and aliquots of the medium were counted every 5 min, up to 45 min, on an Auto Gamma 500 C counting system (Packard, Downers Growe, IL, USA).

## 2.5. Bioassay

Islets of Langerhans were purified from the adult pig pancreas as previously reported [19]. Groups of five islets of uniform size were preincubated for 30 min in 1 ml of krebs-Ringer bicarbonate solution, Hepes 1 mmol, pH 7.4, containing bovine serum albumin (0.5 g/100 ml). After this preincubation period islets were placed inside tubular membranes, previously capped at one end, by simultaneously withdrawing the needle while ejecting the tissue suspension from a plastic syringe. The excess liquid filtered out until appropriate packing was achieved. The membranes were sealed off at the open end to form closed capsules, and the following experiments were performed.

1. Five capsules were separately incubated for 30 min at 37 °C in 1 ml of the krebs-Ringer bicarbonate solution containing 50 and, subsequently 400 mg/100 ml of glucose. At the end of the incubation period the insulin content of the medium was determined. Insulin released from non-encapsulated, freshly isolated islets, which were incubated in the same conditions as the encapsulated one, was used as a control.

2. Three capsules were separately placed for 2 h at 37 °C in 3 ml of the krebs-Ringer bicarbonate solution containing 100 or 400 mg/100 ml of glucose. Aliquots of the medium were assayed every 30 min up to 2 h for their insulin content.

3. Three capsules were separately incubated for 2 h at 37 °C in 3 ml of the krebs-Ringer bicarbonate solution containing 400 mg/100 ml of glucose. The medium content of insulin was measured every 30 min. Insulin released from non-encapsulated islets, incubated in the same conditions as the encapsulated one, was used as a control.

The whole procedure was carried out under sterile conditions.

## 3. Results

The tubular membranes produced in this study were white, opaque, water-swollen spongy material which contained approximately 70–80% water (calculated from the difference between the wet and the dry weights of the specimens). A scanning electron micrograph of the longitudinal section of a membrane capped at one extremity is shown in Fig. 2. The

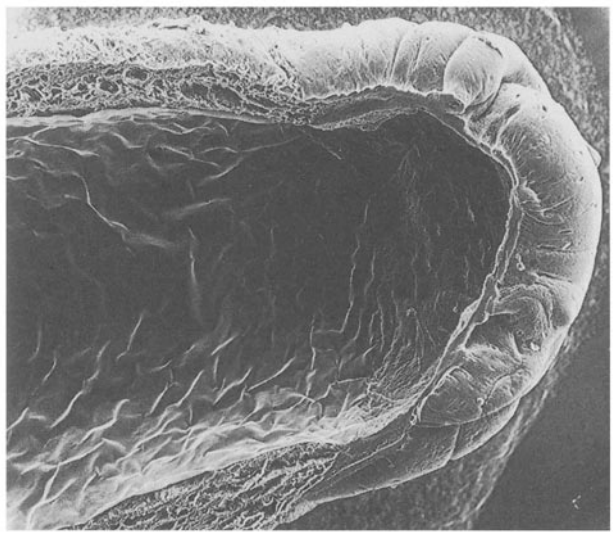

*Figure 2* Scanning electron micrograph of the longitudinal section of a capped PU-PDMS tubular membrane (1.5 mm i.d.). The picture shows the luminal side covered by a dense skin which appears slightly wrinkled (SEM artefact). Note the tight sealing between membrane end and glue cap. Original magnification 50 x.

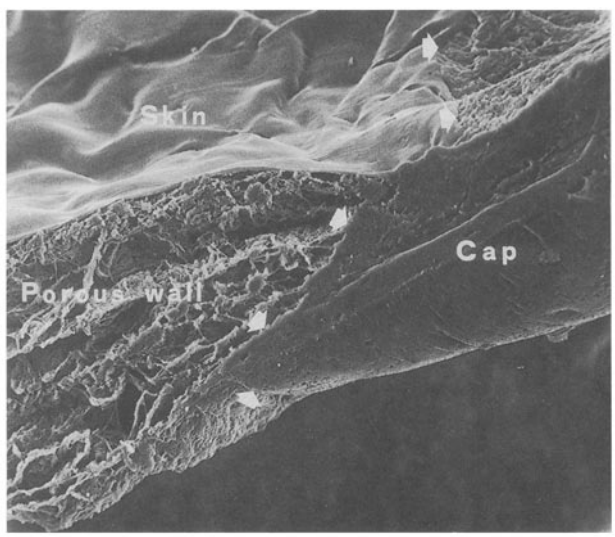


*Figure 3* Scanning electron micrograph of a detail of Fig. 1 showing the border line between the porous wall of the membrane and the dense wall of the cap (arrows). The border line continues on the luminal side between the membrane skin and the cap inner surface (arrows). The thickness of the skin and of the porous wall are approximately 1 and 150 µm respectively. Original magnification 200 x.

membrane material shows a smooth skin on the inner side, and an open trabecular structure in the wall. At higher magnification (Fig. 3) one can better appreciate the thickness of the skin and of the porous wall, which are approximately 1 and 150 μm respectively. In addition, the same picture shows a detail of the area where the membrane and the cap fuse. One may note the border line (arrows) between the porous wall of the membrane and the dense wall of the cap. The border line (arrows) further continues on the luminal side between the membrane skin and the cap's inner surface. A relatively high magnification of the outer surface (Fig. 4) shows an interconnected porous structure with pore size varying from 15 to 80 μm. At the same magnification the inner surface (Fig. 5) shows a dense structure lacking any visible porosity.

The results of the permselectivity studies, performed by GP-HPLC, show that the wall of the membrane is permeable to Vit B12, insulin, and myoglobin (Fig. 6a, b); compounds with a molecular mass of 44 000 Da (ovalbumin) or higher remain inside the capsule. The analysis of the content of the medium inside the capsules, performed by spectrophotometry and light microscopy, shows that IgG and blood white cells do not cross from the external medium into the capsules.

Fig. 7 shows the pattern of glucose diffusion across the capsule wall as a function of time. After 7–8 min of incubation the diffusion reached the plateau and 100% of the glucose had been released out of the capsules. Fig. 8 shows the amount of insulin diffused from the capsules into the medium as a function of time. After 25 min of incubation, more than 80% of the entrapped insulin had been released.

The results of the bioassay are reported as follows.

1. In presence of 50 and 400 mg/100 ml of glucose, the insulin released from encapsulated islet (IRI) was 2.7 ± 0.8 and 4.3 ± 0.7 μunits per islet per minute ($p < 0.05$) respectively (Fig. 9); these figures were not significantly different from those obtained with non-encapsulated tissue.

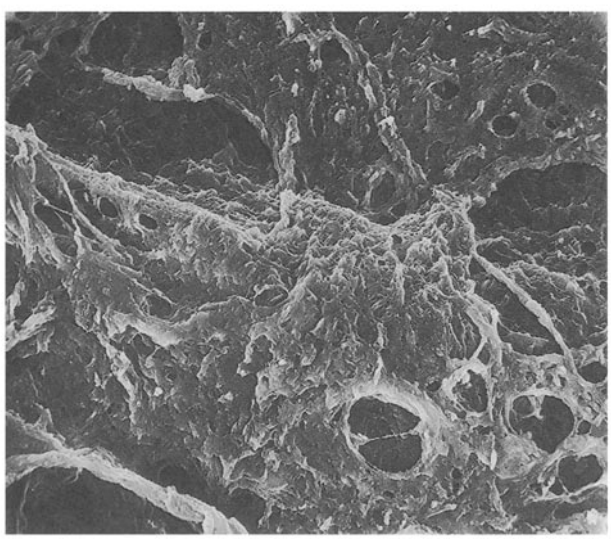

*Figure 4* Scanning electron micrograph of the tubular membrane outer surface showing an interconnected porous structure with pore size of the order of 15–80 μm. Original magnification 500 x.

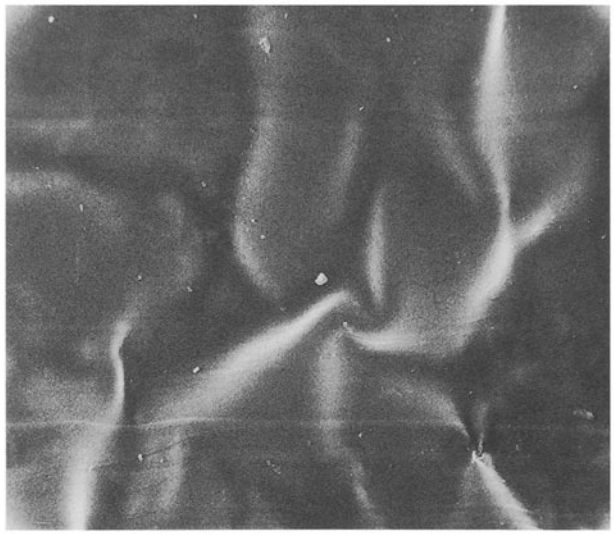

*Figure 5* Scanning electron micrograph of the tubular membrane inner surface showing a dense structure lacking of any visible porosity. Original magnification 500 x.

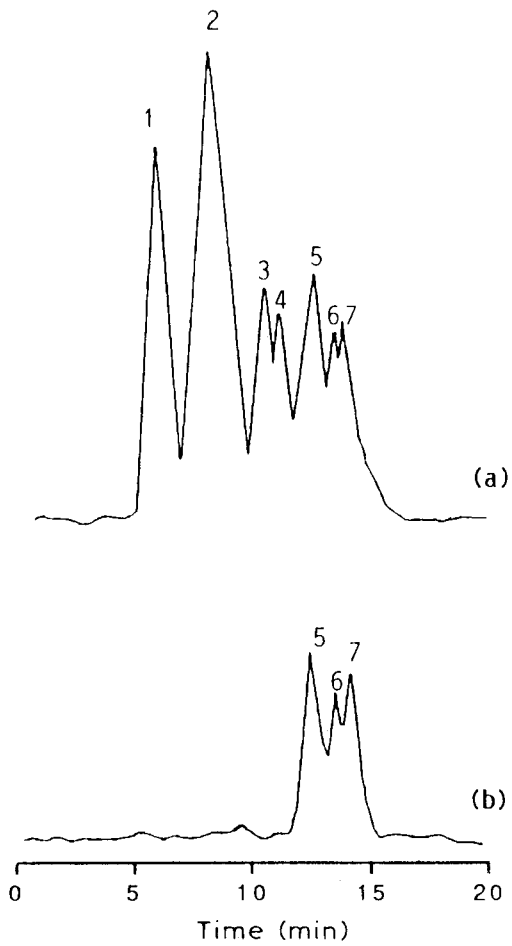


*Figure 6* (a) GP-HPLC profile of a mixture of the reference compounds of different molecular masses listed in Table I. (b) GP-HPLC profile of the medium in which capsules containing a mixture of the reference compounds have been incubated for 30 min. The membrane results permeable to Vit B12 (7), insulin (6), and myoglobin (5).

2. The amount of insulin released from encapsulated islets incubated at 100 or 400 mg/100 ml of glucose as a function of time is shown in Fig. 10. One may note that the output of the hormone was proportional to the concentration of the secretagogue.

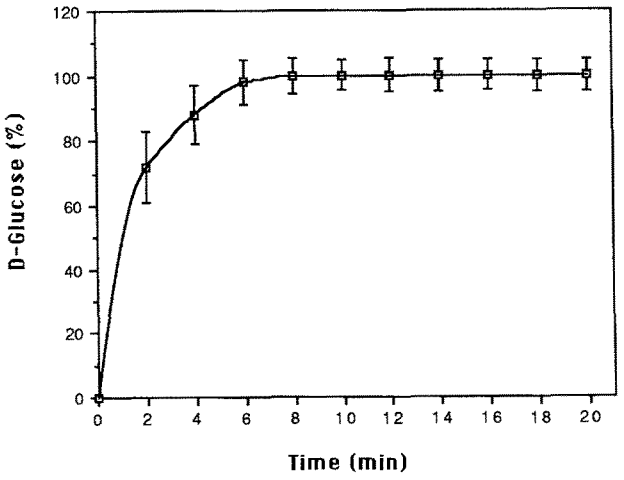


*Figure 7* Diffusion pattern of glucose (%) from the capsule functioning of time. The plot averages data obtained from four diffusion experiments

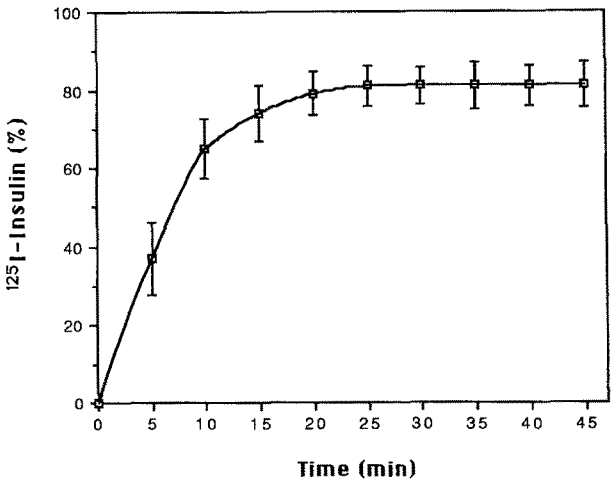


*Figure 8* Diffusion pattern of $^{125}$I-Insulin (%) from the capsule functioning of time. The plot averages data obtained from three diffusion experiments.

3. Finally, encapsulated and non-encapsulated islets released similar amounts of insulin over a 2 h period (Fig. 11)

## 4. Discussion

Transplantation of immuno-protected islet of Langerhans to avoid rejection is an attractive possibility for the successful treatment of the diabetic disease. Among the immuno-protection procedures [3], we focused on the macroencapsulation technique, because of the ease of transplantation and availability of multiple sites for implantation. However, the technique relies on a biomaterial which has to be inert, durable and compatible in a variety of body locations. The biomaterial serves as the skeleton for the functional unit and is typically fabricated as a tube, or an array of tubes, closed at the extremities. The biomaterial is often referred to as a "membrane" because the initial work in this field dealt with monolayer cell cultures. However, progress increasingly demands three-dimensional structures in which the membrane

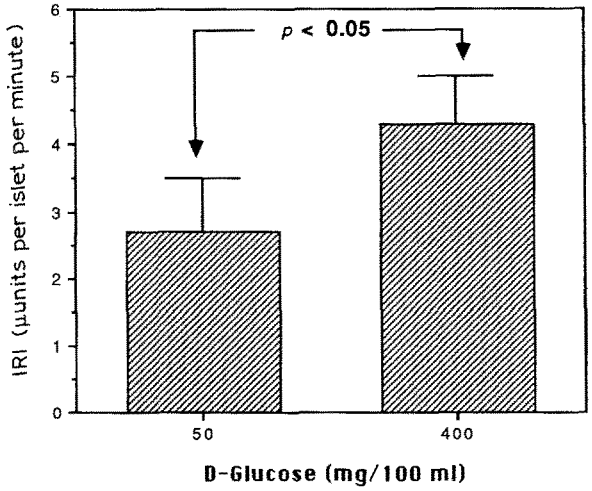


*Figure 9* Insulin released from encapsulated islet (IRI) in presence of 50 and 400 mg/100 ml of glucose after 30 min of incubation time. Insulin is expressed in µunits per islet. Columns average data obtained from five releasing experiments for each glucose concen tration.

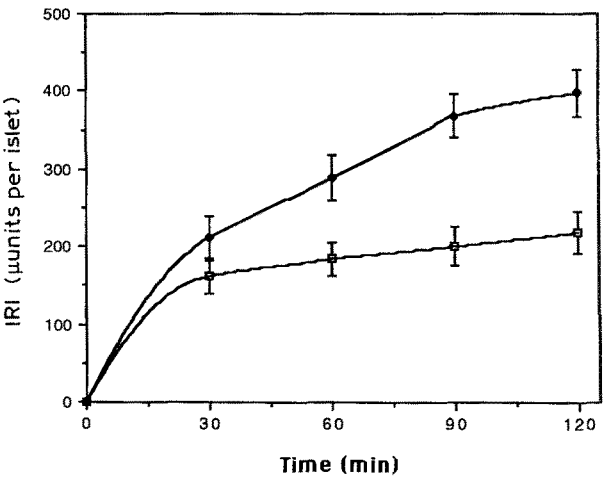


*Figure 10* Insulin released from encapsulated islet (IRI) as a function of time in presence of 100 (□) or 400 (◆) mg/100 ml of glucose. Insulin is expressed in µunits per islet. Plots average data obtained from three releasing experiments for each glucose concentration.

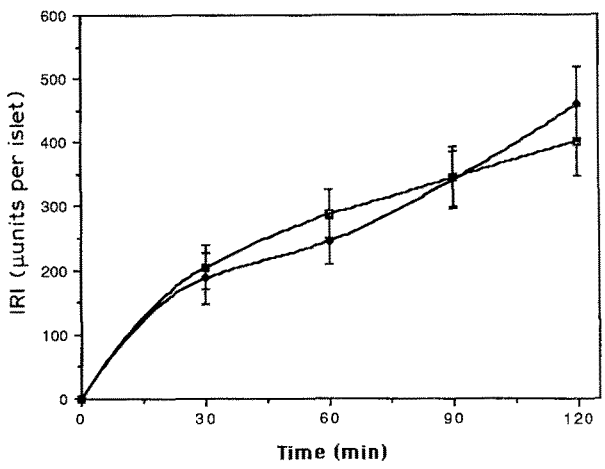


*Figure 11* Insulin released from encapsulated islet (IRI) (□) compared with insulin released from non-encapsulated islet (◆), as function of time and in presence of 400 mg/100 ml of glucose. Insulin is expressed in µunits per islet. Plots average data obtained from three releasing experiments both for encapsulated and non-encapsulated islet

(i.e. the polymer layer which controls the permselectivity) is only a component of the biomaterial.

In this study we fabricated porous tubular membranes obtained by spraying and simultaneously precipitating a PU-PDMS solution over a rotating mandrel. By varying the amount of non-solvent in the polymer solution and adjusting the mechanical parameters of the spinning process, the porosity of the tube wall can be varied over a wide range. In addition, an asymmetric three-dimensional structure can be obtained depositing a non-porous or minimally porous skin at the inner surface of the tube. Capsules made with the PU-PDMS membrane material showed excellent diffusion and permselectivity properties, being permeable to glucose and insulin, but not to compounds with a molecular mass higher than approximately 40 000 Da. Islets, purified from the adult pig pancreas and entrapped in the capsules, released insulin proportionally to the glucose concentration in the medium without a significant difference compared with the amount of insulin released by non-encapsulated islets over a 2 h period.

We conclude that the spraying, phase-inversion process allows the fabrication of asymmetric tubular membranes for cell encapsulation. The PU-PDMS blend appears to be a suitable material for capsule fabrication. It possesses adequate mechanical characteristics for cell filling and its good sealing properties in the wet state may assure good islets immunoprotection. However, the effectiveness of the sealing has to be assessed with *in vivo* experiment. If occlusion between the tube wall and the cap material is not perfect, inflammatory cells or protein molecules which surround the implant will find their way through the interstices and deny to the transplanted tissue the benefits of immuno-isolation [20]. The location of the implant may be an important factor concerning the intensity of the inflammatory reaction around the polymer shell of a bioartificial pancreas unit [20]. In fact, the severity of the host tissue reaction affects the long-term viability of the encapsulated endocrine tissue reducing the rate of solute diffusion to and from the implant. Therefore, the biocompatibility of these capsules will have to be evaluated in a specific experimental model, such as animal species and implantation sites. Nevertheless, the excellent permselectivity and diffusion properties of PU-PDMS capsules, as described in the present report, suggest the possibility of using these capsules for the treatment of human diabetes and possibly other diseases.

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