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A polyurethane–polyvinylpyrrolidone interpenetrating polymer network for mammalian cell encapsulation

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A biocompatible and noncytotoxic interpenetrating polymer network (IPN) membrane was developed for medical applications. The biostable membranes developed could also support the growth and adhesion of mice L929 fibroblast cells. This membrane could, therefore, serve as a candidate material for mammalian cell encapsulation.

THE presently available therapy of type 1 diabetes mellitus mainly involves therapy with insulin injections. However, this form of therapy cannot prevent the nonphysiological fluctuations in blood glucose levels and secondary complications may develop over the years leading to blindness, kidney disease, etc. Success of an alternate therapy such as transplantation of normal insulin-producing cells or islets of Langerhans is limited by immunorejection[1]. Immunorejection may be overcome either by transplanting the islets under heavy doses of toxic immunosuppressive drugs or by immunoisolation[2]. The success of the more desirable immunoisolation therapy would depend on the availability of extremely biocompatible, semipermeable membranes which would prevent the cells of the immunological system from migrating to the encapsulated islet cells and at the same time allow the diffusion of glucose and insulin through the membranes.

Microcapsules of alginate and polylysine have been utilized for encapsulating islets[3]. Limited stability, biocompatibility and permselectivity[4] have prevented their extensive use in humans. Hollow fibres of acrylates, cellulose acetate, etc., have been used with limited success[5] to encapsulate the islets. The main drawbacks limiting their use appear to be fragility and biocompatibility. The most significant development in this area was made by Calafiore *et al.*[6], who encapsulated islet cells in alginate-polylysine membranes and further entrapped the encapsulated cells within the walls of a PTFE vascular prosthesis. Permeability control, biocompatibility and optimum mechanical properties can be better achieved by implanting the cells in polyurethane membranes.

Polyurethanes are extensively used in biomedical applications but suffer from degradative processes in long-term applications. Techniques of interpenetrating polymer network synthesis have been utilized to achieve better resistant polyurethanes in long-term applications. We have carried out the interpenetration of polyurethane networks with both hydrophilic networks such as polyacrylamide[7], polyvinylpyrrolidone[8], polyhydroxyethylmethacrylate[9] and hydrophobic network such as polymethylmethacrylate[10] to obtain better resistant polyurethanes. Extensive *in vitro*[11] and *in vivo*[12] studies carried out for these IPNs demonstrated the biocompatible and biostable nature for many of the compositions. This study highlights the application of a polyurethane–polyvinylpyrrolidone membrane for cell encapsulation.

Synthesis and characterization of the polyurethane (PU) and the polyurethane–polyvinylpyrrolidone interpenetrating polymer network (PU–PVP IPN) have been reported elsewhere[7,8]. Characterization studies of the IPNs involving studies on chemical resistance, mechanical behaviour, spectroscopy, dynamic mechanical behaviour, surface hydrophilicity and morphology characteristics by scanning electron microscopy were all carried out using standard procedures. Biostability of the PU and IPN membranes was assessed by monitoring the changes in the mechanical properties of the membranes subsequent to implantation in rats. Cytotoxicity of the synthesized membranes was evaluated using an indirect contact test based on a tetrazolium dye (MTT) assay[13].

The membranes were assessed for their cell growth support characteristics by fixing them on tissue culture Petri dishes using a medical-grade silastic adhesive (Dow corning). The growth of L929 fibroblast cells in contact with the membranes was assessed by comparing the cell shape, appearance and spread relative to that of control grown in similar Petri dishes, by visual observance through a microscope.

The biocompatibility aspects of the membranes were also assessed by implanting them intramuscularly in black-hooded Liverpool strain Lister rats for periods of one month and three months. After explanation, the tissues around the implants were stained by an immunostaining method[14] that used monoclonal antibodies for specifically staining the ED2 macrophages. Specific stains were also employed to stain B cells, T cells and neutrophils. The cell number and distribution were quantified using a computer-aided image analysis system.

Characterization results are summarized in (Table 1). Mechanical properties of the IPN are consistent with the behaviour of a typical viscoelastic material. Shift of the loss ($\tan\delta$) peak or glass transition temperature of the IPN to a higher temperature than that obtained for the homopolymer polyurethane is indicative of

Table 1. Physicochemical characterization

Sample	Mechanical properties: Tensile strength (MPa)	Mechanical properties: Elongation (%) at break	Mechanical properties: Modulus at 100% elongation	DMA studies: tan δ°C	Contact angle: θ air	Contact angle: θ octane
PU	14 12	746	5 69	–22	53 6	115 0
PU–PVP IPN	6 51	448	3 92	10	40 0	135 0

Table 2. Changes in mechanical properties on implantation

	Decrease in tensile strength (%)	Increase of elongation (%)
PU	20	0
PU–PVP IPN	1	22

enhanced mixing or compatibility of the IPN. The decrease of air–water contact angle for the PU–PVP IPN over that of the polyurethane is an index of the higher surface hydrophilicity obtained on IPN formation. In general, a hydrophilic surface promotes better compatibility to the vascular tissues[15].

The chemical resistance studies have shown that the PU–PVP IPN with even 90 parts polyurethane and 10 parts polyvinylpyrrolidone could withstand degradation in a solvent such as dioxan, which is a very good solvent for the polyurethane network, emphasizing the enhanced chemical resistance obtained on IPN formation.

The decrease in tensile stress with practically no change in elongation for the polyurethane homopolymer upon implantation in rats (Table 2) is suggestive of a degradative mechanism in which the polyurethane undergoes chain scissions in a hostile physiological environment. However, on IPN formation, the decrease in tensile stress is considerably reduced, indicating the better biostability achieved on IPN formation. The accompanying increased elongation has been attributed to a plasticizing action of water and biological molecules in the physiological environment.

An important aspect of prescreening new polymers of biomedical potential is to establish their general cytotoxicity to cell culture *in vitro*. In the indirect contact test[13], tetrazolium salts (MTT) are reduced to equivalent formazan precipitates by live and metabolically active cells.

The activity of the L929 fibroblast cells when incubated with the extracts of the polyurethane and IPN for a period of 24 h was compared with the activity of a homogeneous L929 control cell population. The percentage activity obtained for cells incubated with IPN extracts was 74%, which is suggestive of a noncytotoxic behaviour[13]. The percentage activity of the cells incubated with the polyurethane extract was only 17%, indicating cytotoxicity and confirming the biodegradative nature of this material.

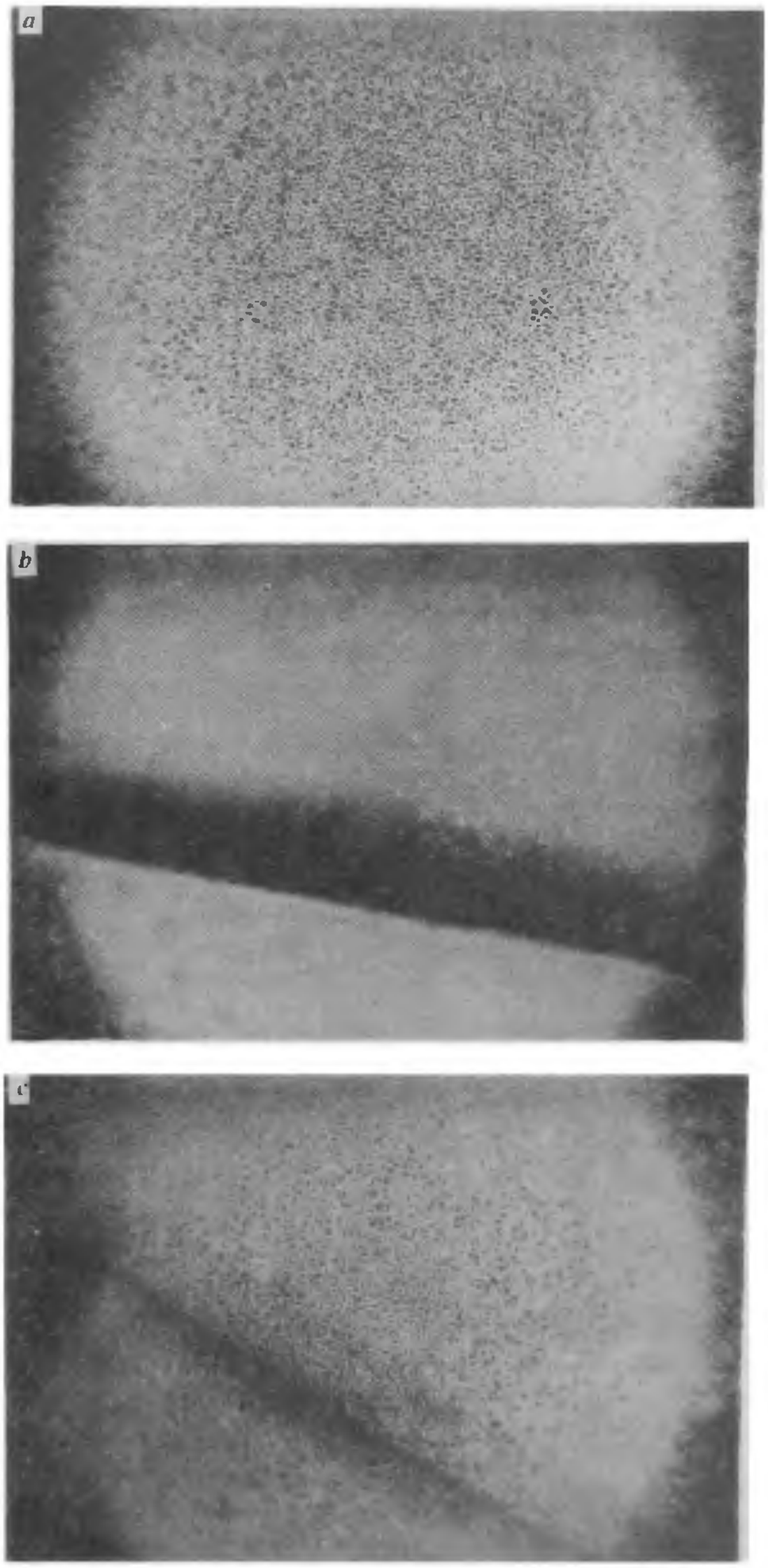

Figure 1. L929 fibroblast cells in culture *a*, control cells, *b*, cells in contact with the PU membrane, *c*, cells in contact with the PU–PVP IPN membrane (×40)

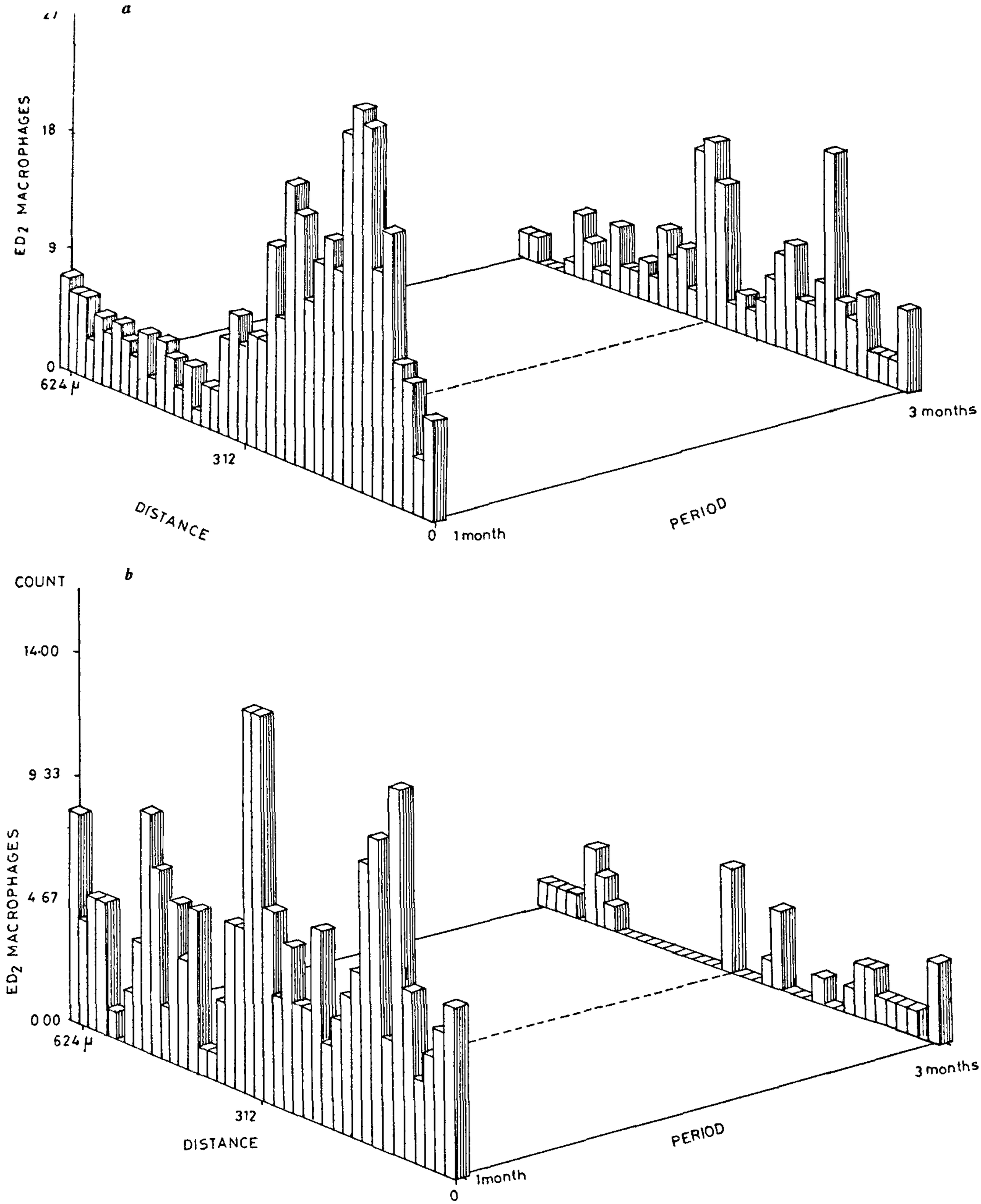

Figure 2. Distribution of the ED2 macrophages at the implant site as a function of time and distance: *a*, PU membrane; *b*, PU–PVP IPN membrane.

Microphotographs of the L929 fibroblast cells grown in contact with the polyurethane (PU) and the PU–PVP IPN as well as control cells are depicted in Figure 1 *a–c*. The control cells (Figure 1 *a*) are spindle-shaped, uniformly and thickly spread out, and have a glistening appearance characteristic of a normal and healthy condition. The cells in contact with the degrading PU membrane (Figure 1 *b*) have lost their spindle shape and become round, indicating cell lysis. Some of the cells were observed floating in the medium. The PU membrane used in this case is definitely not supportive of cell growth or adhesion. Figure 1 *c* depicts the L929 cells in contact with the IPN membranes. The profuse cell growth that is uniformly spread as in the control cell population is clearly seen. The spindle shape and the glistening appearance of the cells are also evident. These studies therefore indicate that by IPN formation, the degrading PU membranes could be made more bioresistant and highly supportive of cell growth and adhesion. This IPN membrane may, therefore, be eminently suitable for encapsulation of living cells in the fabrication of artificial internal hybrid organs such as artificial pancreas and liver.

Clinical indication of any deficiency in biocompatibility as far as the performance of a material is concerned is best brought out by studies of local tissue response. Histological studies[12] of the local tissue response on implanting the PU and PU–PVP IPN membranes had indicated better compatibility for the IPN. This response was further confirmed by quantifying the response using specific immunostaining techniques and computer-aided image analysis[14].

The image analysis studies, utilizing monoclonal antibodies for specific staining, could recognize the absence of T cells, B cells and neutrophils and the predominance of ED2 macrophages. Macrophages are known to play several roles in the inflammatory response. One of the roles of the macrophage is to phagocytose cellular and molecular debris and also to detoxify and/or sequester toxic materials.

The polyurethane membrane induced an initial inflammatory response evidenced by a maximum number of ED2 macrophages, i.e. 366, in one month. With the stabilization of the response at three months, the macrophage number dropped down to 161. The distribution of these macrophages even at the end of 3 months was uniformly spread out and higher at the implant interface (Figure 2 *a*).

In contrast, the ED2 macrophages were 210 in number at one month at the IPN interface and this had dropped to a mere 21 isolated cells in the vicinity of the implant (Figure 2 *b*), at the end of 3 months. The IPN membrane can, therefore, be considered as a very biocompatible material.

In conclusion, interpenetrating polymer networks of polyurethane have been synthesized with desired physicochemical properties. The materials are, therefore, ideal candidate materials for encapsulating pancreatic cells or hepatocytes for the fabrication of artificial internal hybrid organs. Ongoing work in progress is concerned with establishing the permeability characteristics of these membranes to metabolites and other components of extra cellular matrix for effective functioning of the hybrid organ.

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Fractal relation of perimeter to the water body area

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The relation between the fractal dimension of the perimeter and the area of a water body is given. The fractal dimension of the water bodies arrived through perimeter–area relationship is tallied with their actual fractal dimensions. The fractal dimension of the water bodies under study is very close to that of the Brownian mountain lakes.

THE relation of water body area A to the perimeter P of the water body is one of the important hydrologic