The influence of polymeric membrane surface free energy on cell metabolic functions

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In membrane bioartificial organs using isolated cells, polymeric semipermeable membranes are used as immunoselective barriers, means for cell oxygenation and also as substrata for adhesion of anchorage-dependent cells. The selection of cytocompatible membranes that promote *in vitro* cell adhesion and function could be dependent on its membrane properties. In this study we investigated the physicochemical aspects of the interaction between the membrane and mammalian cells in order to provide guidelines to the selection of cytocompatible membranes. We evaluated the metabolic behavior of isolated liver cells cultured on various polymeric membranes such as the ones modified by protein adsorption. The physico-chemical properties of the membranes were characterized by contact angle measurements. The surface free energy of membranes and their different parameters acid (γ^+), base (γ^-) and Lifshitz-van der Waals (γ^{LW}) were calculated according to Good-van Oss's model. The adsorption of protein modified markedly both contact angle and membrane surface tension. In particular, membrane surface free energy decreased drastically with increased water contact angle. For each investigated membrane we observed that liver specific functions of cells improve on hydrophilic membrane surfaces. For all investigated membranes the rate of ammonia elimination increased with increasing of membrane surface free energy.

1. Introduction

In bioartificial organs using isolated cells, polymeric semipermeable membranes are used as immunoselective barriers between patient's blood and the xenocytes to prevent rejection [1]. Membranes also provide a large exchange area to supply cells with amounts of nutrients and oxygen necessary for their metabolism [2]. In some devices as in the case of bioartificial liver, membranes also act as the substrata for cell adhesion, the hepatocytes being anchorage-dependent cells [3].

Most mammalian cells must be supported by a tissue specific extracellular matrix, which plays an essential role in the cell proliferation and the maintenance of tissue functions of a large number of organs and tissues. *In vitro* the same mechanical and chemical support must be provided to cell culture microenvironment. The capacity of a membrane to perform a support function for cell culture depends on its surface properties. Surface free energy, electric charge and morphology might all affect cell attachment and its behavior either indirectly, e.g. by controlling adsorption of the proteins present in the culture medium (or secreted by the cells), or directly, e.g. by guiding cell spreading with suitable surface topography [4, 5]. As a result, such properties play a critical role in cell-substratum interaction and have to be considered in the selection of membranes suitable for biomedical devices.

Previously we showed that the morphology of hepatocytes adherent to a substratum changes with its properties and that liver cells interact better with rougher and wettable membranes in comparison to non-wettable and smooth membranes [6–7]. The basis for this difference is still poorly understood but may be explained in part by variation of the amounts and conformation of adhesion proteins, which adsorb on native substratum contacting cells. Measurements of the wettability of membrane, expressed by the contact angle in the presence of different liquids permit to evaluate and to compare surface free energy of membranes with different physico-chemical properties. Such measurements before and after modification of native membranes in culture medium might be predictive indices of their

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TABLE I Contact angles and surface free energy parameters of different polymeric membranes

Membrane	θ DIM [°]	θ W[°]	θ Gl [°]	γ^{LW}[mJ m^{-2}]	γ^- [mJ m^{-2}]	γ^+ [mJ m^{-2}]	γ^{AB} [mJ m^{-2}]	γ[mJ m^{-2}]
PP	98.2 ± 5	140 ± 3.7	145 ± 3.4	9.4	0.36	3.06	2.1	11.4
PSf	62 ± 1.2	81 ± 2.5	73 ± 1.6	27.4	7.4	0.5	3.8	31.3
PC	53 ± 1.5	76 ± 7.1	73 ± 3.5	32.3	10.2	0.07	1.7	33.9
PAN	65.2 ± 2	50.7 ± 5	54.2 ± 1.9	33.8	12.96	1.64	9.22	43

cytocompatibility and/or tissue biocompatibility. Therefore, a material surface treatment might enable the adaptation of its surface free energy to biological requirements.

This study was performed to understand the role of surface free energy of different polymeric membranes in the interaction with liver cells. The cell metabolic functions on membranes with different surface free energy were compared as parameters of cytocompatibility. Since serum proteins modify membrane surface properties, the surface free energy of membranes were tested before and after modification into culture medium containing serum proteins. Considering that hepatocytes in bioartificial liver are primarily used to detoxify blood from neurotoxic species (e.g. ammonia) the ability of liver cells to eliminate ammonia was investigated [8].

2. Materials and methods

2.1. Membrane characterization

Four flat sheet microporous membranes with different physico-chemical properties were used: polycarbonate (PC) (Cyclopore-Whatman, MA, USA); polysulfone (PSf) (Dow Liquid Separations, Cheshire, England); polyacrilonitrile (PAN) membranes (Membrane Products Kiryat Weizmann LTD, Rehovot, Israel) and polypropylene (PP) (Enka AG, Wuppertal, Germany). Membranes were sterilized with 2% (w/v) glutaraldehyde and then extensively rinsed with sterile double distilled water.

The membranes were characterized by contact angle measurements. Contact angle of various test liquid droplets on the membrane surfaces were measured by sessile drop method at ambient temperature by using CAM 200 contact angle meter (KSV Instruments LTD, Helsinki, Finland). The sessile drop was formed by depositing the used test liquid from above using an automatic microsyringe on the membrane surfaces. Three reference liquids (distilled water, diiodomethane and glycerol) were used to determine the apolar γ^{LW}, the acid-base γ^{ab}, acid (electron acceptor) γ^+, base (electron donor) γ^-, components of surface free energy by means the method of Good *et al.* [9].

The surface tension and their components of the test used liquids were taken from literature. Results are the mean of six measurements of different regions of the sample surface. All measurements were repeated four times. To avoid cross contamination of liquids a dedicated microsyringe was used for each liquid.

2.2. Protein adsorption

The membranes were incubated in minimum essential medium eagle containing 10% (v/v) fetal calf serum (Gibco BRL, Paisley, UK) at 37 °C for different times. Each incubation was performed using quadruplicate samples in order to allow monitoring variability in the extent of protein adsorption onto any single membrane sample. The proteins adsorbed on the surface were removed from the sample and were determined by protein assay using bicinchoninic acid solution (Sigma, St. Louis, MO, USA). The experimental data are presented as the amount of protein adsorbed per unit surface area (cm^2) of polymeric membranes. All membranes modified by protein adsorption were characterized by contact angle measurements.

2.3. Hepatocyte isolation and culture

Hepatocytes were isolated from livers of adult male Wistar rats according to the method by Berry and Friend, as modified by Seglen, and described elsewhere [10]. Cell viability after isolation was determined by trypan blue exclusion [11]. The isolated hepatocytes were seeded on the membranes to give a surface concentration of 7×10^4 cells cm^{-2} and were incubated in minimum essential medium (Eagle) supplemented with 10% fetal calf serum (Gibco BRL, Paisley, UK), 50 μg ml^{-1} gentamicin sulfate, 10 μM insulin and 1 μM dexamethasone. The cultures were incubated at 37 °C in a 5% CO_2 : 21% O_2 atmosphere (the balance being N_2).

Hepatocyte functions were assessed by means of initial velocity measurements at every change of the culture medium by incubating the hepatocyte cultures and controls with MEM added with 1 mM NH_4Cl for 2 h at 37 °C [12]. The metabolic rates of ammonia elimination were estimated by accounting for the correction of controls. Ammonia concentration was assayed by the enzymatic method (Sigma, St. Louis, MO, USA).

The statistical significance of the experimental results was established according to Student's *t*-test.

3. Results and discussion

The investigated membranes have different hydrophobic/hydrophilic characteristics, as can be seen in Table I, where the results of the membrane contact angle are reported. The contact angles and the components of membrane surface free energy vary by changing the type of polymer. The surface free energy γ calculated according to Good-van Oss's approach, consisting of the sum of the Lifshitz-van der Waals γ^{LW} term and the acid and base term γ^{ab}. For PC, PAN and PSf the base parameter (γ^-) is much bigger compared to the small acid parameter (γ^+), on the contrary, PP membranes have small base parameter. All investigated membranes exhibited different values of surface free energies with a trend PAN > PC > PSf > PP.

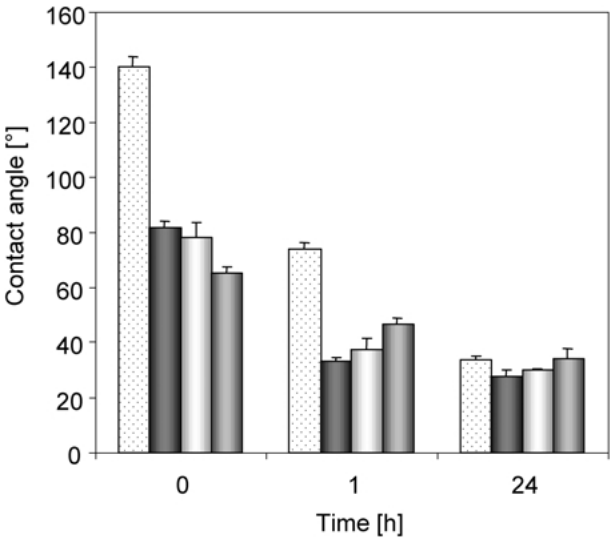

Figure 1 Changes in contact angle of various membranes after adsorption of serum proteins at different time: PP membrane; PSf membrane; PC membrane; PAN membrane.

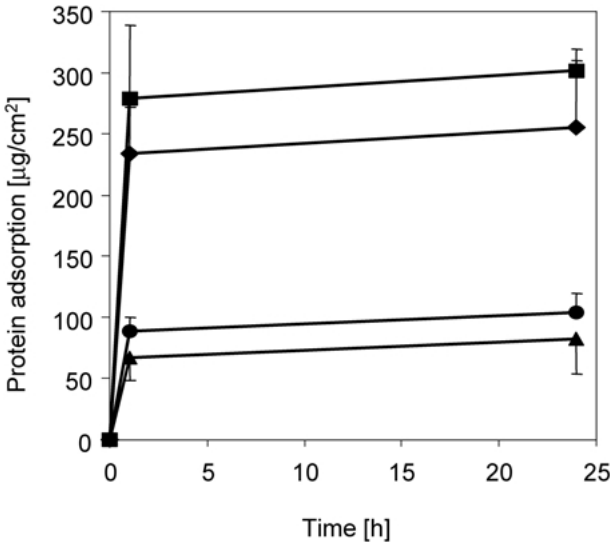

Figure 3 Extent of protein adsorption on different membranes after different time of incubation: (■) PP membrane; (◆) PC membrane; (●) PSf membrane; (▲) PAN membrane.

The native physico-chemical characteristics of membrane surface were modified in order to obtain different wettable surfaces for each type of membrane. Serum proteins contained in the culture medium modified the values of membrane contact angles and surface free energy. As is shown in Fig. 1, the adsorption of proteins modified markedly contact angles. The membrane contact angle decreased with time of incubation into culture medium: after 24 h of incubation, the contact angle value for PP, PSF and PAN membranes is about half with respect to the initial value. The membrane surface free energy decreased drastically with increased water contact angle as is depicted in Fig. 2. The adsorption of proteins was dependent on the chemical nature of native membranes and on the incubation time (Fig. 3). The greatest amount of adsorption occurred on those membranes with the low surface free energy 11 mJ m^{-2}. In fact on PP membranes the amount of proteins adsorbed on the surface was 280 and 300 µg cm^{-2} after respectively 24 and 48 h. Also on PC membranes with contact angle of 78° the amount of adsorbed proteins is higher than that observed on more hydrophilic membrane surfaces. This pattern of adsorption is in agreement with previously reported experimental data with respect to flat sheet polymers [13]. Two interesting points emerge from consideration of experimental data. The first one is that the extent of protein adsorption is relatively large on hydrophobic membranes and relatively small on more hydrophilic membranes. There is one exception: PSf membrane although being more hydrophobic than PC membrane adsorbed fewer proteins than PC membrane. Secondly, on each of the four membranes examined, the protein adsorption resulted in an increase of membrane surface free energy and of hydrophilicity.

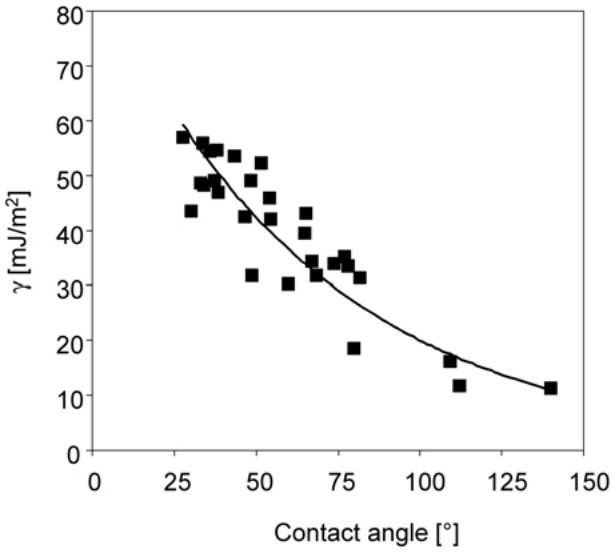

Figure 2 Changes in surface free energy of membrane related to contact angle after adsorption of serum proteins.

The native and modified membranes were used for liver cell culture. The metabolic activity of cells was affected to a large extent by membrane wettability. For each investigated membrane cells eliminated ammonia with a rate that increased with increasing of membrane surface free energy. The metabolic activity is particularly expressed at high levels when cells were cultured on membranes with surface free energy ranging 48–57 mJ m^{-2}. Fig. 4a shows that on PC membranes the ability of liver cells to eliminate ammonia increased linearly with increasing the value of surface free energy and reached maximal value in correspondence of the surfaces with $\gamma = 49$ mJ m^{-2}. On such membrane surfaces, cells exhibited rates of ammonia elimination of 13 pg h^{-1} $cell^{-1}$ that is significantly higher than that of cells cultured on the same type of polymeric membrane but on surface with $\gamma = 34$ mJ m^{-2}. On PSf membranes the rate of ammonia elimination increased with surface free energy reaching a maximal value of 42 pg h^{-1} $cell^{-1}$ on surface with $\gamma = 57$ mJ m^{-2} (Fig. 4b). Values of ammonia elimination rate were not significantly different when cells were cultured on PSf membranes with lower surface free energy than 53 mJm^{-2}. When cells were cultured on PAN membranes, the cell metabolic activity increased weakly with membrane surface free energy (Fig. 4c); however, highest rates of ammonia elimination were measured on PAN membranes with high surface free energy. Also

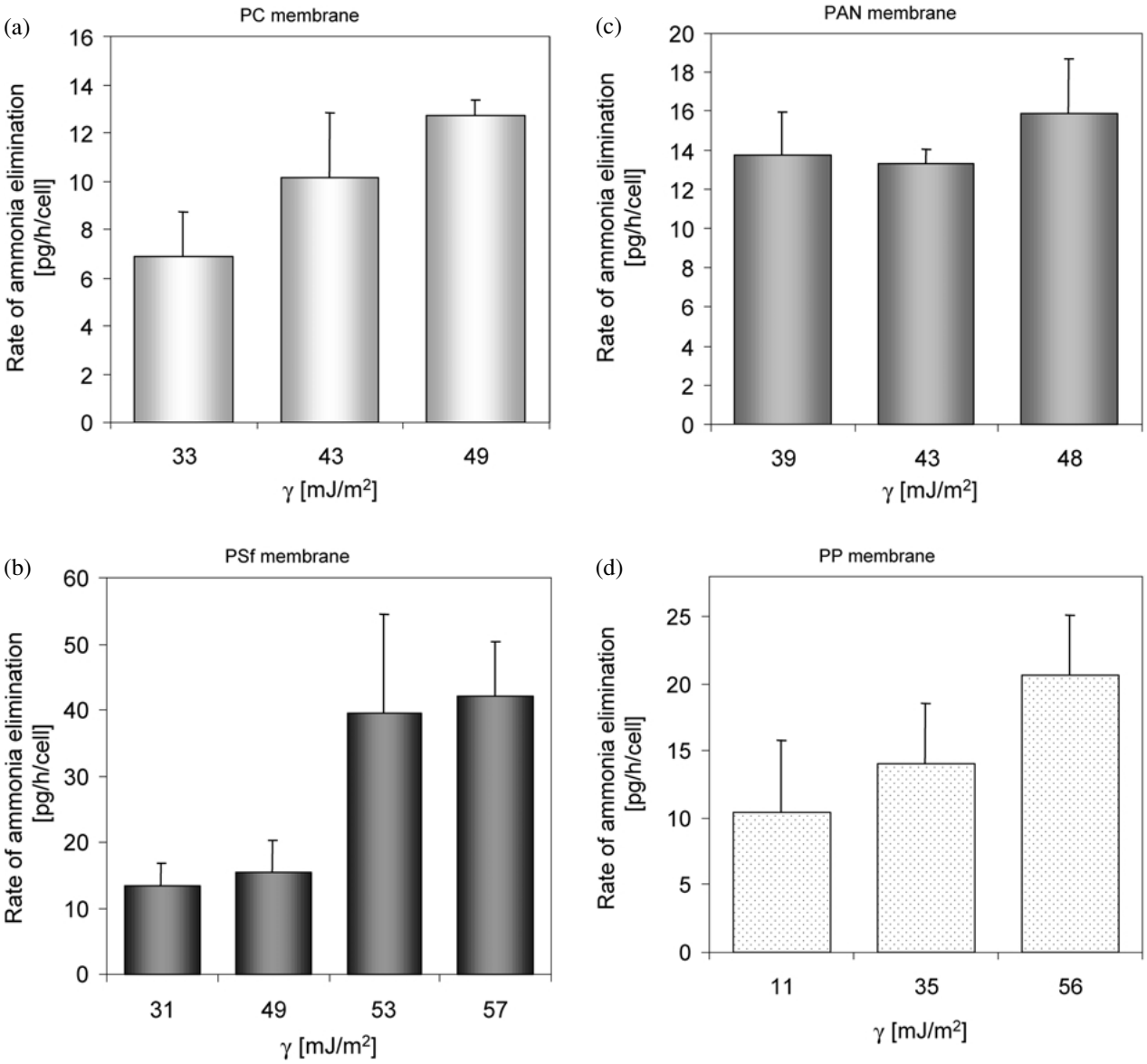

Figure 4 Rate of ammonia elimination of liver cells cultured on membranes with different surface free energy: (a) PC membrane; (b) PSf membrane; (c) PAN membrane; (d) PP membrane.

on native hydrophobic membrane such as PP membranes the functional behavior of cells in terms of ammonia elimination improved with increasing the wettability of surfaces (Fig. 4d). Other authors observed a relationship between adhesion of different type of cells and different wettability of materials: when substratum surface free energies were low, cell adhesion was poor [14,15]. A better cell adhesion could lead to a better metabolic state of cells.

These findings show a relationship between ammonia elimination rate of liver cells and membrane surface free energy. Independently on the type of the native polymeric membranes it is possible to improve cell specific functions by changing its surface free energy.

4. Conclusions

The results demonstrated that the cell-membrane interaction of the four investigated membranes is improved by changing its surface free energy. The membrane surface free energy was modified by protein adsorption, which produced for each polymeric membrane surfaces with different wettability. Maximum protein adsorption occurs on membranes with lowest surface free energy, corresponding to the most hydrophobic. For all investigated membranes, a rise in the surface free energy promoted liver specific functions of cells. Enhancement of the ammonia elimination rate is related to the membrane surface free energy of the investigated membranes.

Our results suggest that it is possible to improve the cytocompatibility of the membrane surface by changing its physico-chemical surface characteristics. However, the surface free energy is only one of the indicators of cell behavior on membranes. Other surface parameters such as surface charge or the chemical structure of a polymer can also have an effect on the overall behavior of tissue on polymeric membranes.

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