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New modified polyetheretherketone membrane for liver cell culture in biohybrid systems: adhesion and specific functions of isolated hepatocytes

L. De Bartolo*, S. Morelli, M. Rende, A. Gordano, E. Drioli

Institute on Membrane Technology, National Research Council of Italy, ITM-CNR, c/o University of Calabria, via P. Bucci cubo 17/C, Rende, Cosenza (CS) I-87036, Italy

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Abstract

There has been growing interest in innovative materials with physico-chemical properties that provide improved blood/cell compatibility. We propose new polymeric membranes made of modified polyetheretherketone (PEEK-WC) as materials with potential for use in biohybrid devices. PEEK-WC exhibits high chemical, thermal stability and mechanical resistance. Owing to its lack of crystallinity this polymer can be used for preparing membranes with cheap and flexible methods.

We compared the properties of PEEK-WC membranes to polyurethane membranes prepared using the same phase inverse technique and commercial membranes. The physico-chemical properties of the membranes were characterised by contact angle measurements. The different parameters acid (γ^+), base (γ^-) and Lifshitz-van der Waals (γ^{LW}) of the surface free energy were calculated according to Good-van Oss's model.

We evaluated the cytocompatibility of PEEK-WC membranes by culturing hepatocytes isolated from rat liver. Cell adhesion and metabolic behaviour in terms of ammonia elimination, urea synthesis and protein synthesis were evaluated during the first days of culture. Liver cells adhered and formed three-dimensional aggregates on the most tested membranes. PEEK-WC membranes promoted hepatocyte adhesion most effectively. Urea synthesis, ammonia elimination and protein synthesis improved significantly when cells adhered to PEEK-WC membrane. The considerable metabolic activities of cells cultured on this membrane confirmed the good structural and physico-chemical properties of the PEEK-WC membrane that could be a promising biomaterial for cell culture in biohybrid devices.

Keywords: PEEK-WC membrane; Hepatocytes; Surface free energy; Adhesion; Functions

1. Introduction

Synthetic membranes in fibre and flat configurations for their functional characteristics like selective permeability and stability are used in different biomedical devices contacting blood and cells [1,2]. In bioartificial organs (i.e., liver, pancreas) membranes act as immunoprotective barriers and also play a role of mechanical and chemical support for adhesion and growth of cells. Membranes with adequate mass transport and surface properties favouring interactions with cells are required. Several studies have shown the importance of the morphological and physico-chemical properties of the polymer surface in cell interactions [3,4]. Surface free energy, electric charge and morphology might all affect cell attachment and behaviour either indirectly, e.g., by controlling adsorption of the proteins or directly, e.g., by guiding cell spreading with suitable surface topography. It has been shown that the morphology of hepatocytes adhering to a substratum changes with properties (roughness, wettability, surface free energy parameters) and that maintenance of cell morphology similar to that in the natural liver is important for hepatocyte function [5–7].

Most of the membranes commercially available were developed for blood treatment and were adapted to cell culture but with limited success. In fact, different functional characteristics are required of membranes when they are used in blood devices and in biohybrid systems. In this respect, modification of traditional

*Corresponding author. Tel.: +39-0984-492010; fax: +39-0984-402103.
E-mail address: l.debartolo@itm.cnr.it (L. De Bartolo).

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polymeric materials has been proposed to improve the cytocompatibility of conventional materials [8,9]. Significant efforts have also been made to develop membranes with better cell interactions. In this paper, we propose new polymeric membranes made of modified polyetheretherketone (PEEK-WC) or poly(oxa-1,4-phenylene-oxo-1,4-phenylene-oxa-1,4-phenylene-3,3-(isobenzofurane-1,3-dihydro-1-oxo)-diyl-1,4-phenylene) as materials with potential for use for cell culture in biohybrid systems. Membranes were prepared from PEEK-WC, which exhibits chemical stability and excellent thermal and mechanical resistance similarly to traditional PEEKs, which are used in medical implants. Differently from PEEKs, PEEK-WC is soluble in various solvents owing to lack of crystallinity. This characteristic allows its use for preparing membranes by phase inversion, which is a cheap and flexible method [10]. Previous studies have shown that this novel membrane is very promising for various applications [11,12]. The developed PEEK-WC membranes combine the advantageous properties of the polymer with those of membranes such as permeability, selectivity and stability.

In this study we compared the interactions of liver cells with PEEK-WC membrane to commercial membranes and polyurethane (PU) membranes prepared using the same phase inverse technique. The physico-chemical properties of PEEK-WC and PU membranes were compared with those of commercial membranes used conventionally in biomedical applications. We evaluated the cytocompatibility of membranes by assessing adhesion and liver specific functions of isolated rat hepatocytes. Primary isolated mammalian hepatocytes from differing cell lines and genetically engineered cells express all normal differentiated functions as in vivo and are useful for various studies [13]. Considering that hepatocytes in a bioartificial liver are primarily used to detoxify blood from neuroactive species, as well as ammonia, endogenous and exogenous compounds, we investigated the ability of cells to synthesise urea and to eliminate ammonia in the presence of ammonia in the culture medium [14]. Protein synthesis of hepatocytes adhering to membranes were also assessed.

In order to evaluate the ability of PEEK-WC membranes to support the adhesion and liver specific functions of cells, we related the metabolic rates to physico-chemical parameters of membrane surfaces.

2. Materials and methods

2.1. Membranes

We investigated two novel modified polyetheretherketone membranes (PEEK-WC20 and PEEK-WC60) made in our laboratory by inverse phase technique using the direct immersion-precipitation method. PEEK-WC solutions in DMF were mixed to PVP at 80/20 (PEEK-WC20) and 40/60 wt% (PEEK-WC60), respectively. The dopes were cast by hand-knife and coagulated in a water bath at room temperature. The membranes were washed extensively with water and dried at room temperature [12]. In a similar way PU membranes were prepared from solid medical grade Pellethane® 2363-80AE (Dow Chemical Company, Netherlands). Polymeric solutions at 9 wt% in *N*,*N*-dimethylformamide (DMF) of PU were mixed at 50°C with polyvinilpyrrolidone (PVP) in the mass ratio of PU/PVP 1.4/1. After casting the polymeric films were coagulated in a bath of DMF/EtOH 20/80 v/v. Thereafter membranes were washed extensively with water and dried at room temperature.

As commercial membranes we used three flat sheet microporous membranes with different physico-chemical properties: Nylon (NY) (Osmonics, USA), Polyethersulphone (PES) (Membrana GmbH, Wuppertal, Germany), Polyester (PETE) (Osmonics, USA).

2.2. Membrane characterisation

The wettability properties of all membranes were characterised by using the water contact angle measurements with dynamic and sessile drop methods at ambient temperature using a CAM 200 contact angle metre (KSV Instruments LTD, Helsinki, Finland). The physico-chemical properties of all the membranes were characterised by using the Good van Oss approach. This approach gives the possibility of estimating an acid–base contribution to the solid surface tensions and allows the determination of attractive and repulsive interactions [15]. The contact angle of various test liquid droplets on the membrane surfaces were measured by sessile drop depositing the used test liquid from above using an automatic microsyringe on the membrane surfaces. Three reference liquids (ultrapure water, diiodomethane and glycerol) were used to determine the apolar γ^{LW}, the acid–base γ^{AB}, the acid (electron acceptor) γ^{+}, the base (electron donor) γ^{-} and the components of surface free energy by means of Good, van Oss and Chaudhury's method [16]. The surface tension of the three reference liquids was measured by the pendant drop method. The Lifshitz-van der Waals component γ^{LW} of the membrane surface tension reflecting the dipole interactions was calculated from the measured diiodomethane contact angles under the assumption that diiodomethane is an apolar test liquid:

$$\gamma_s^{LW} = \frac{\gamma_l^{LW}(1+\cos\theta)^2}{4},$$

where subscripts s and l indicated, respectively, solid and liquid.

After the γ^{LW} of membrane surface has been measured, it is possible to calculate the other components (γ^{AB}, γ^- and γ^+) by using two polar liquids, such as glycerol and water:

$$\gamma_l(1+\cos\theta) = 2\left(\sqrt{\gamma_s^{LW}\gamma_l^{LW}} + \sqrt{\gamma_s^+\gamma_l^-} + \sqrt{\gamma_s^-\gamma_l^+}\right)$$

and

$$\gamma_s^{AB} = 2\sqrt{\gamma_s^-\gamma_s^+}.$$

The surface tension and components of the liquids used in the test were taken from literature [14].

The results are the mean of ten measurements of different regions of the sample surface. All the measurements were repeated six times. To avoid cross-contamination of liquids a microsyringe was dedicated to each liquid.

The roughness of the membrane surfaces prepared in our laboratory was evaluated by using Atomic Force Microscopy, Nanoscope III (Digital Instruments, VEECO Metrology Group). Tapping Mode™ AFM operated by scanning a tip attached to the end of an oscillating cantilever across the sample surface. The cantilever was oscillated at or near its resonance frequency with an amplitude ranging typically from 20 to 100 nm. Silicon probes were used. Surface roughness was estimated with respect to the mean absolute value difference, R_a, and the root mean squared difference, RMS, between the actual surface height and that of the line dividing the surface of the investigated profile into two equal areas. The reported roughness values are the average of 20 measurements on different membrane samples, according to the American National Standard Institute/American Society of Mechanical Engineers standard. The specimens were analysed without any particular treatment.

2.3. Hepatocyte isolation and culture

Hepatocytes were isolated from the livers of adult male Wistar rats according to the method used by Berry and Friend, as modified by Seglen, and described elsewhere [17]. Cell viability after isolation was determined by trypan blue exclusion. The isolated hepatocytes were seeded on the membranes lying at the bottom of polystyrene Petri dishes to give a surface concentration of 7×10^4 cells/cm^2 and were incubated in minimum essential medium (Eagle) supplemented with 10% foetal calf serum (Gibco BRL, Paisley, UK), 50 μg/ml gentamicin sulphate, 10 μM insulin and 1 μM dexamethasone. A medium with foetal calf serum was used only for the first day of culture. Thereafter, a medium without serum was used. The cultures were incubated at 37°C in a 5% CO_2: 21% O_2 atmosphere with 95% relative humidity (the balance being N_2). Collagen coated Petri dishes were used as reference substrata. Type I lyophilised collagen from rat tail (Roche Diagnostics, Mannheim, Germany) was dissolved with sterile acetic acid to give a final concentration of 2 mg/ml. Collagen solution was added to the dishes in order to obtain a coating concentration of 5 μg/cm^2.

The morphological changes of liver cells adhering to the membrane surface were analysed by observation of the samples under scanning electron microscopy (SEM). The cell surface concentration on the different substrata was determined by DNA measurements after 24 and 48 h.

The physico-chemical properties of the isolated hepatocytes were characterised by using the method described by van Oss et al. [18], which consists in contact angle measurements done on a flat layer of cells. After isolation, the initial cell suspension was filtered onto a microporous support (e.g., cellulose acetate, Millipore) with a pore diameter of 1 μm in order to obtain a complete layer of cells covering the support. Thereafter, the cell layer was dried to avoid any influence of initial cell geometry and it was assessed for the contact angle in water, glycerol and diiodomethane. All the measurements were taken only on hepatocyte suspension fresh from isolation. Each measurement was repeated ten times and for three different isolation experiments. The surface free energy parameters of the hepatocytes were determined by means of the above-mentioned Good van Oss equations.

Hepatocyte functions in terms of ammonia elimination, urea synthesis and proteins synthesis were estimated by means of initial velocity measurements at every change of the culture medium. The rate of ammonia elimination and urea synthesis were assessed by incubating the hepatocyte cultures and controls with medium added with 1 mM NH_4Cl for 2 h at 37°C [14]. The metabolic rates were estimated by accounting for the correction of the controls.

2.3.1. Biochemical assays

Samples of the culture medium were collected from cell cultures and controls in pre-chilled tubes and stored at −20°C until assayed. The protein content in the samples was determined by protein assay using bicinchoninic acid solution (Sigma, St. Louis, MO, USA) by spectrophotometer analysis. The ammonia concentration was estimated within 2 h of collection according to the enzymatic L-glutamate dehydrogenase assay (Sigma, St. Louis, MO, USA). The urea concentration was assayed by the enzymatic urease method (Sigma, St. Louis, MO, USA).

For DNA analysis, the cells were detached from the membrane by the addition of 0.05% collagenase in PBS followed by incubation at 37°C for 30 min. The cells were centrifuged for 10 min at 2500 rpm. The cell pellet was washed twice with 10 ml PBS and re-suspended in nuclear lyses buffer (10 mM Tris-HCl pH 8.0, 400 mM

NaCl, 2 mM EDTA pH 8.0, 1 mg/ml proteinase K). After incubation at 39°C overnight, samples were precipitated in saturated NaCl solution (6 M) and centrifuged at 2500 rpm for 15 min. DNA was then extracted after the addition of 100% ethanol. The DNA was washed with ETOH 70% (v/v) and re-suspended in TE buffer (0.01 M Tris-HCl, pH 8.0 and 0.001 M EDTA, pH 8.0) [19]. The DNA was measured by spectrophotometer assay using diphenylamine.

The statistical significance of the experimental results was established according to the Unpaired Statistical Student's *t*-test ($p < 0.05$).

2.4. Sample preparation for SEM

Specimens of cell cultures were prepared for SEM by fixation in 2.5% glutaraldehyde, pH 7.4 phosphate buffer, followed by post-fixation in 1% osmium tetroxide and by progressive dehydration in ethanol [20]. The specimens were examined by SEM after plating with gold under vacuum.

3. Results

The contact angle measurements of various test liquids on investigated membranes are summarised in Table 1. The energy parameters: acid, base, acid–base and Lifshitz Van der Waals parameters of the membrane surface free energy demonstrated the actual surface physico-chemical characteristics of the membranes. The investigated membranes have a high energy surface with $\gamma > 40\,mJ/m^2$ and are mainly electron-donors. For PEEK-WC20 and PEEK-WC60 membranes as well as for other commercial membranes such as PES, NY and PETE membranes, the base parameter (γ^-) is much greater compared to the low acid parameter (γ^+). PU membranes appear to be both electron-donors and electron-acceptors.

In Fig. 1 advancing and receding contact angles are reported as a function of the membrane type. Advancing contact angles through static contact angle measurements confirmed the increase in wettability of the membrane surfaces in the following sequence: NY > PES > PEEK−WC20 ≅ PEEK−WC60 > PETE > PU. Both PEEK-WC membranes were more wettable than PU membranes. The hysteresis of contact angle measured on the investigated membranes could be affected by the surface rearrangement and reorganisation of chemical groups on the surface as well as by the surface roughness of the membrane. Table 2 shows the values of R_a and RMS of all the membrane surfaces. The membranes exhibited different surface roughness. The effect of roughness on wettability can be observed in the case of the PEEK-WC membranes where the difference of contact angles measured between the two surfaces is due to the different roughness of the membranes: PEEK-WC20 with $R_a = 3.78 \pm 0.02$ and PEEK-WC60 with $R_a = 2.92 \pm 0.05$ nm.

Considering that the surface energy of the cells is the quantity that affects the interactions of cells with other surfaces and cells, we assessed the physicochemical properties of the hepatocytes after isolation from the liver (Table 3). A layer of fresh isolated hepatocytes have a surface free energy value of $36\,mJ/m^2$ and a base

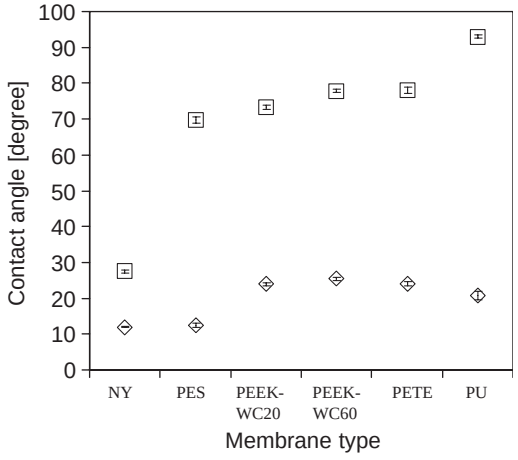

Fig. 1. Advancing and receding contact angle of the novel and commercial membranes.

Table 1
Contact angles in water (W), glycerol (Gly) diiodomethane (DIM) and calculated surface tension parameters of different polymeric membranes: γ^{LW} = Lifshitz-van der Waals component, γ^{-+} = electron acceptor (acid parameter) component and γ^- = electron donor (base parameter) component of the liquid surface tension γ

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)
NY	24±2	49±2	75±2	47	57	4	29	75
PETE	41±2	75±2	81±1	39	15	0.9	72	67
PES	30.4±1	54±1	69±2	44	41	1	14	58
PU	35±3.6	94.4±1.4	100.9±1.6	42.0	9.0	5.52	14.1	56
PEEK-WC-20	24.6±2.4	69.9±2.2	69.5±1.0	46.3	15.7	0.32	4.5	51
PEEK-WC-60	26.2±2.5	71.2±2.8	68.4±0.9	45.7	13.7	0.15	2.9	49
Collagen	52±1.3	82±2.2	67±7.6	33.4	2.3	1.1	3.2	37

The surface tension and its parameters were calculated according to the Good van Oss equation by average values of measured contact angles.

parameter of surface free energy of 70 mJ/m^2. The high value of γ^- demonstrated the presence of mainly electron-donor sites on the cell cytoplasmatic membrane.

After characterisation, isolated rat hepatocytes were cultured on the investigated membranes. During the first days of culture, the liver cells formed three-dimensional aggregates on most of the tested membranes (Figs. 2a–f). The cell aggregates on PEEK-WC20 (Fig. 2a) and PEEK-WC60 (Fig. 2b) membranes are quite larger than those observed on other membranes. Also on PES and NY membranes cells formed large aggregates. Small-flattened aggregates were also formed on the PU membranes.

Cell adhesion on the membranes was quantified by measurements of the DNA content. As is shown in Fig. 3, after 24 h the DNA did not change significantly with membrane surface free energy. After 2 days of culture the DNA concentration peaked on PEEK-WC60 and PEEK-WC20 membranes reaching values, respectively, of 9.5 and 7.3 μg/cm^2. When cells were cultured on PU membranes and on other commercial membranes DNA concentration decreased significantly (Fig. 3).

Consistently with the DNA content, also the liver specific functions of the cells increased as the membrane surface free energy passing through maximal values corresponding to 49–51 mJ/m^2 was increased. Liver cells cultured on PEEK-WC20 and PEEK-WC60 membranes exhibited high metabolic rates (Figs. 4 and 5). After 24 h of culture, the ability of liver cells to eliminate ammonia on PEEK-WC60 and PEEK-WC20 was, respectively, 2- and 2.4-fold greater than those measured on NY. Also on collagen the rate of ammonia elimination was lower by 30% and 40% than that measured, respectively, on PEEK-WC60 and PEEK-WC20 (Fig. 4). On PU membranes the cells exhibited a rate of ammonia elimination of 14.8 ± 0.3 ng/h/cell, which was comparable to that obtained on PETE and PES membranes. Although, after 48 h of culture the ammonia elimination rate decreased on all membranes, on PEEK-WC20 membrane the cells are able to eliminate ammonia with rates 10% and 20% greater than those measured, respectively, on collagen and on other tested membranes.

Similarly, also the urea synthesis rate of hepatocytes significantly increased when the cells were cultured on PEEK-WC60 and PEEK-WC20 membranes (Fig. 5). In particular urea synthesis was expressed at high levels when the cells were cultured on PEEK-WC20 membrane. The ability of cells to synthesize urea decreased drastically when the cells were cultured on other membranes. The urea synthesis rate of hepatocytes cultured on collagen was 2.4-fold lower than values obtained on PEEK-WCs membranes.

The protein synthesis of hepatocytes was also high on PEEK-WCs membranes, as is illustrated in Fig. 6. After 24 h of culture, the higher values of protein synthesis were measured on PEEK-WC60, PEEK-WC20, PU and PES membranes. The ability of cells to synthesise proteins after 48 h of culture did not change on PEEK-WC60 and increased, respectively, by 36% and 40% when cells were cultured on PEEK-WC20 and PES membranes.

Table 2
Surface morphological features of the membranes used in this investigation

Membrane	R_a (nm)	RMS (nm)
NY	88.84 ± 8.8	107.7 ± 9.5
PETE	11.03 ± 1.12	12.55 ± 1.6
PES	14.82 ± 0.85	18.14 ± 1.19
PU	5.92 ± 0.42	7.64 ± 0.64
PEEK-WC20	3.78 ± 0.02	4.75 ± 0.30
PEEK-WC60	2.92 ± 0.05	3.68 ± 0.22

Values are reported as the average ± standard deviation of 60 measurements for each membrane surface.

4. Discussion

In this study the ability of novel PEEK-WC membranes to support liver cell adhesion and functions was evaluated and related to membrane surface free energy. To this purpose we used isolated primary liver cells because the favoured cells for bioartificial liver with respect to cell lines, which express some liver specific functions at different levels [21] are considered.

The contact angles, surface free energy and its parameters calculated on the basis of Good van Oss model evidence the different physico-chemical properties of the investigated polymeric membranes (Table 1). PEEK-WC and PU membranes as well as most of the

Table 3
Contact angles in water (W), glycerol (Gly) diiodomethane (DIM) and calculated surface tension parameters of isolated hepatocytes: γ^{LW} = Lifshitz-van der Waals component, γ^{+} = electron acceptor (acid parameter) component and γ^- = electron donor (base parameter) component of the liquid surface tension γ

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)
68 ± 2	46 ± 2	76 ± 3	24	70	0.2	12	36

The surface tension and its parameters were calculated according to the Good van Oss equation from the average values of measured contact angles.

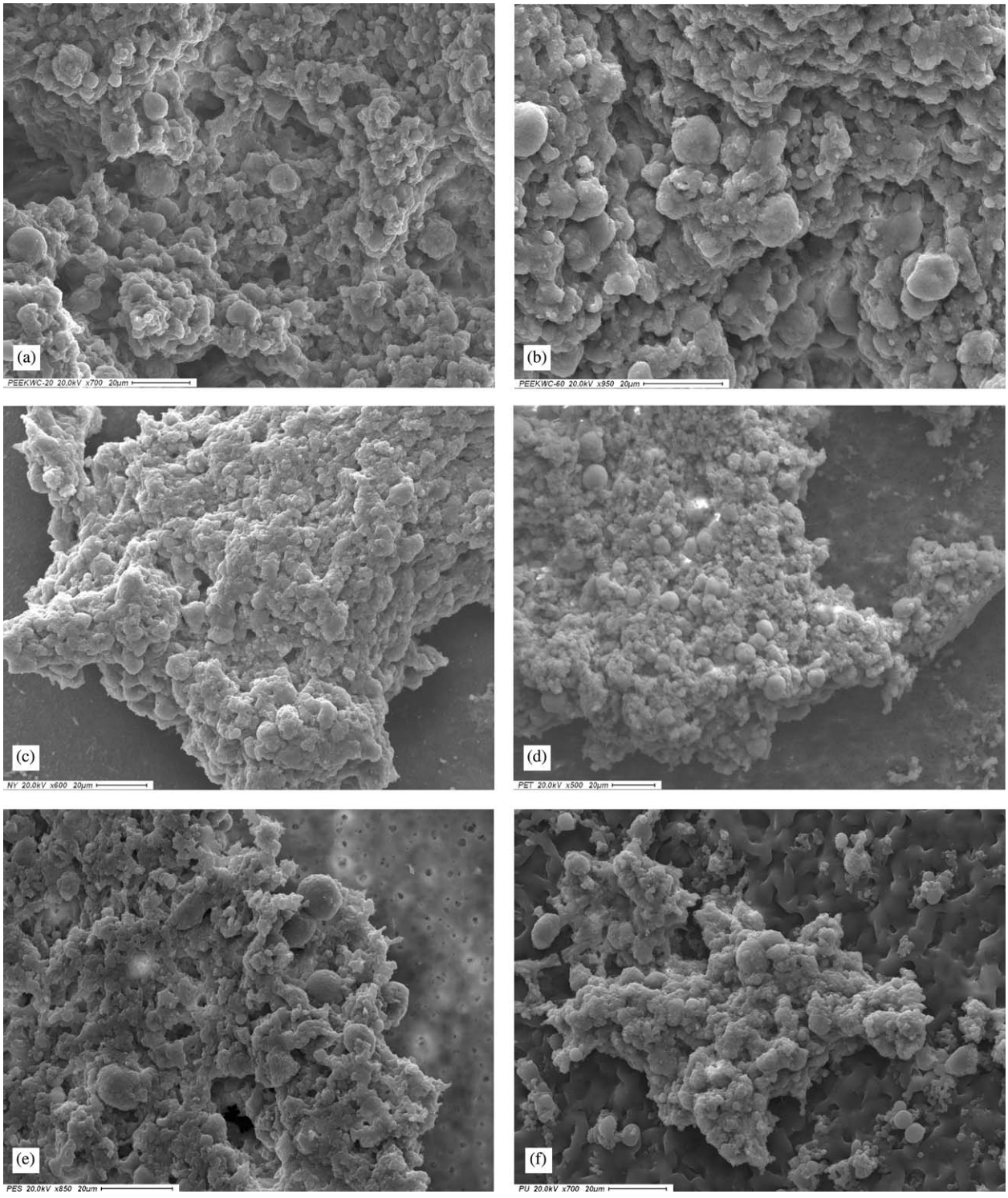

Fig. 2. SEMs observation of liver cells after 48 h of culture on: (a) PEEK-WC20, (b) PEEK-WC60, (c) NY, (d) PETE, (e) PES, and (f) PU membrane.

commercial membranes have a much greater base parameter of surface free energy compared to small acid parameter. The oxygen atoms contained in the polymer as ether and carbonylic functionalities constitute effective Lewis base sites [22]. A comparison of the base parameter of PEEK-WC membranes to that of the other membranes evidenced the moderate hydrophilic character of such membranes with respect to NY (highest base surface character) surface. The moderate hydrophilicity of PEEK-WC surfaces was confirmed by the wettability experiments (Fig. 1). The hysteresis, meaning the difference between advancing and receding

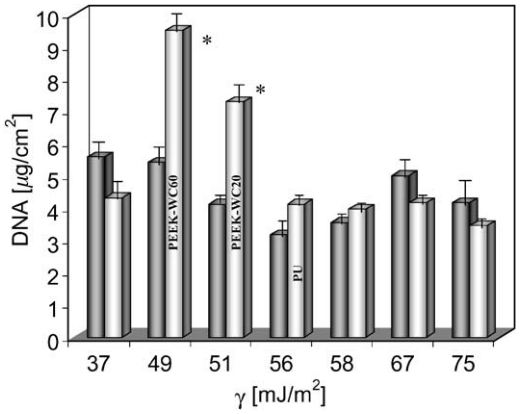

Fig. 3. DNA content after 24 (full bar) and 48 h (shade bar) of culture on membranes with different surface free energy. The DNA values are the mean of eight experiments: ±standard deviation. *Data significant statistically compared to other membranes ($p<0.05$).

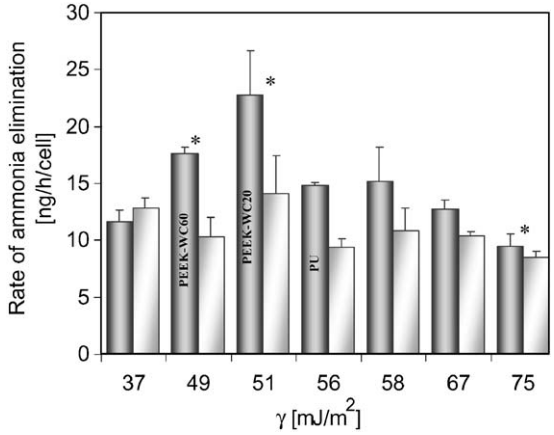

Fig. 4. Ammonia elimination rate of liver cells cultured on membranes with different surface free energy after 24 h (full bar) and 48 h (shade bar) of culture. The metabolic rates were determined by culturing cells in the presence of 1 mM ammonia in the medium. The reported values are the mean of eight experiments: ±standard deviation. *Data significant statistically compared to other membranes ($p<0.05$).

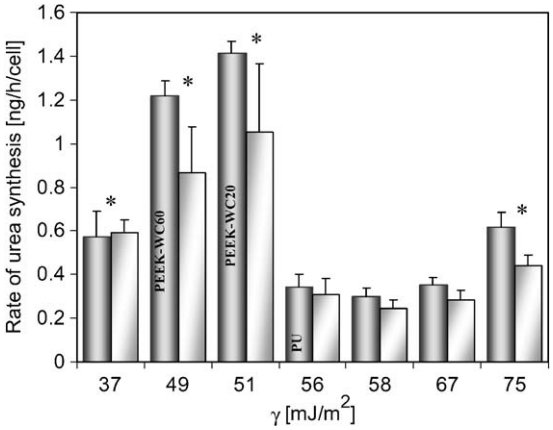

Fig. 5. Urea synthesis rate of liver cells cultured on membranes with different surface free energy after 24 h (full bar) and 48 h (shade bar) of culture. The metabolic rates were determined by culturing cells in the presence of 1 mM ammonia in the medium. The reported values are the mean of eight experiments: ±standard deviation. *Data significant statistically compared to other membranes ($p<0.05$).

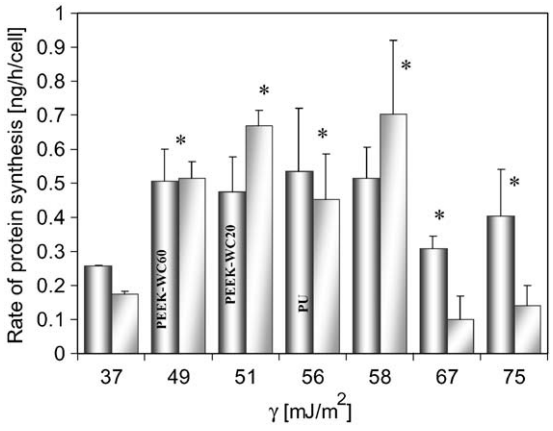

Fig. 6. Rate of protein synthesis of liver cells cultured on membranes with different surface free energy after 24 h (full bar) and 48 h (shade bar) of culture. The metabolic rates were determined by culturing cells without serum in the medium. The reported values are the mean of eight experiments: ±standard deviation. *Data significant statistically compared to other membranes ($p<0.05$).

contact angle, indicated the chemical and topographical heterogeneities which imply that the investigated membranes were real materials. The effect of membrane roughness on the measured contact angle was observed in the case of PEEK-WC membranes which had surfaces with the same chemical composition. However, the difference in the water contact angle of the two PEEK-WC membranes was only 1° confirming the key role of surface chemistry in the determination of wettability.

Figs. 2a–f show that the hepatocytes adhering to PEEK-WC membranes formed cellular aggregates to a large extent, as with NY and PETE membranes. The three-dimensional organisation of hepatocytes is important in maintaining the cell functions. In fact, tissue architecture and cell morphology are crucial for the proper functioning of cells [2–23] within the organ in situ. In vitro studies have shown that hepatocytes cultured in a three-dimensional system in both batch and flow conditions maintained stable levels of albumin and drug metabolisation [2,14]. Instead cells adhered less efficiently to PU membranes. The large cell aggregation observed on PEEK-WC membranes is consistent with the peak of DNA content found on the same membranes after 48 h of culture, as shown in Fig. 3. This behaviour is probably dependent on surface chemistry and on the protein layer formed on the membrane surface. As cell surface free energy parameter data also demonstrated (Table 3), hepatocytes have many electron-donor sites present on the glycoproteins

of the cellular membrane, which are responsible for cell attachment and the organisation of an extracellular matrix [24]. The electron-donor sites on the cell glycoproteins could bind to electron-acceptor sites present on the polymer or could be attracted by electrostatic forces to a negatively or positively charged interface by some linkers such as Ca^{2+}, Mg^{2+} and protein bridges like fibronectin, vitronectin and laminin. The presence of linkers could neutralise the electron-donor monopolar energy and favour the establishment of cell–cell and cell–surface contacts. Clearly this leads to the replacement of cell–water bonds with interactions between the membrane molecules of the interacting surfaces, which may be viewed as partial dehydration driven by the mutual attraction of interacting surfaces. Furthermore, it was demonstrated for fibroblasts that cell adhesion involved a re-organisation of adsorbed fibronectin, which occurred on hydrophilic surfaces and not on a hydrophobic surface [25]. Probably PEEK-WC membranes tend to adsorb adhesive proteins from the surroundings, which could have a beneficial effect on the attachment and functions of the cells. These results are in agreement with our previous observation by using membranes modified by protein adsorption [7] when an increase in cell adhesion and functions was found on membranes with high energy parameters.

This morphological behaviour affected metabolic functions in terms of ammonia elimination, urea synthesis and protein synthesis. The metabolic activity is particularly expressed at high levels when cells are cultured on PEEK-WC20 and PEEK-WC60 membranes. Figs. 4 and 5 point out that on such membranes cells show enhanced functional activity in terms of ammonia elimination and urea synthesis. Cells eliminated ammonia with a rate that increased as the membrane surface free energy passing through maximal values of 49–51 mJ/m^2 increased. A similar functional behaviour was observed concerning the urea synthesis on PEEK-WC20 and PEEK-WC60. An increase respectively of 60% and 70% of the urea synthesis rate after 24 h of culture was measured on PEEK-WC20 and PEEK-WC60 with respect to the other membranes. Although the ability of cells cultured on these membranes to synthesise urea decreased with time, after 48 h of culture cells retained their metabolic activity at values from 0.9 to 1.1 ng/h/cell, which are higher than those exhibited by cells on other membranes.

Differently from ammonia elimination and urea synthesis, the ability of cells to synthesis proteins was high on PEEK-WC, PU and PES membranes. Furthermore, on PEEK-WC and PES membranes the protein synthesis rate increased after 48 h of culture. The reorganisation of cells in aggregates seems to have a positive impact on some functional features of cells such as their ability to synthesise proteins. This effect has been observed also by other authors [26]. Tight cell–cell contacts in aggregates would provide better conditions for protein synthesis functions.

On the basis of morphological and functional data, the PEEK-WC membranes proved to be surfaces that favoured interactions with cells in terms of protein synthesis, urea synthesis and ammonia elimination. This behaviour is related to the good physico-chemical properties of the PEEK-WC membrane that support the adhesion and liver specific functions of the primary hepatocytes, which are considered the favoured cells for biohybrid liver systems.

5. Conclusions

PEEK-WC membranes proved to be surfaces that favour cell adhesion and expression of their metabolic activities. Liver cell behaviour on membranes was dependent on the physico-chemical properties of the membranes. A relationship between the membrane surface free energy and metabolic activities was found. Liver specific functions of liver cells measured in terms of ammonia elimination and urea synthesis rates increased as the wettability of membranes passing through maximal values increased, which corresponded to the membrane surface free energy of PEEK-WC membranes. The moderate wettability of PEEK-WC surfaces was responsible for improving interfacial interactions between the membrane and liver cells.

PEEK-WC membranes owing to their easy manufacture, their intrinsic polymeric physico-chemical characteristics and their interesting surface properties, seem to be promising biomaterials. The results obtained from liver cell culture experiments encourage further investigations in order to evaluate the full potential of these membranes for use in bioartificial organs.

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