

PLASMA INDUCED GRAFTING OF STYRENE AND (2-CHLOROETHYL) VINYL ETHER ONTO POLYETHYLENE II. ASSESSMENT OF BIOCOMPATIBILITY BY HEPATOCYTES

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ABSTRACT

High density polyethylene (HDPE) sheets were treated in a commercial barrel-type reactor by an argon RF glow discharge and grafted by subsequent exposure to (2-chloroethyl)vinyl ether and styrene vapor, respectively. The changes of chemical composition, morphology, and surface energy are reported in another paper of this conference [1]. The biocompatibility of the polymer surfaces obtained was assessed by cultures of rat liver cells (hepatocytes) using commercial tissue culture plastic as a standard.

The supportive capacity of HDPE could be considerably improved by modifying its surface with styrene, while it was deteriorated by corresponding modification with (2-chloroethyl)vinyl ether. Styrene-grafted HDPE is even more compatible than tissue culture plastic usually used for the cultivation of hepatocytes.

INTRODUCTION

Because of its unique opportunities to modify even chemically inert surfaces, low temperature plasma techniques have attracted attention by scientists involved in biocompatibility research [2,3]. Already at an early stage, *plasma treatment* using oxygen, nitrogen, and ammonia plasmas was used to increase the wettability of plastics for improved cell adhesion by fixing a variety of O- and N-containing functional groups on the polymer surface [4,5]. Strong interest focussed on *plasma polymerization* because it makes it possible to create layers with unique properties on nearly any solid material [3,6,7,8]. On the other hand, the "structural scrambling" [3] of the monomer causes far less defined polymer structures as can be seen by the variety of chemical environments indicated by the C1s envelope of the ESCA spectra [3]. Furthermore, it cannot be excluded that a significant portion of low molecular weight material is

incorporated in the plasma polymer layer that may slowly leak out especially if the deposition was done under "soft" plasma conditions (low W/FM values) [9].

Recently, however, it was stated that so far "most biomaterials exhibit a nonspecific biological reaction" and postulated that it is more reasonable to "synthesize precisely engineered surfaces in order to direct specific processes" [10].

Similar to radiation induced graft copolymerization, *plasma induced grafting* [11] causes less complex reactions on the substrate compared to plasma oxidation [2], if a noble gas plasma is used. Since the grafting step is performed in the absence of a plasma, no "scrambling" of the monomer takes place. Instead, it can be expected that the grafted side chains form by a specific radical chain reaction as known from ordinary polymer chemistry.

Thus, plasma induced grafting may have the potential to prepare "surfaces for this new generation of materials" that are "precisely defined (with each atom in an engineered position or spatial range), at equilibrium, and resistant to contamination" "in contrast to our present materials which are amorphous or polycrystalline, drift in structure and composition with time, and suffer from uncontrolled contamination" [10].

It was shown by ESCA and AFM, that Ar plasma induced graft copolymerization creates some roughened but branched and maybe crosslinked surface of HDPE. Some oxidation with the oxygen singly bound to the carbon also occurred. Grafted styrene and (2-chloroethyl)vinyl ether (CE) were fixed to the HDPE as polymers in shape of randomly oriented fibrils [1]. So, in contrast to plasma polymerization, obviously some long range ordered, probably crystalline polymer (in the classical meaning) with some loose and open structure was synthesized. A sodium signal from the HDPE substrate having approximately 7% of its former intensity indicates that the base material was covered only to roughly 93% by the grafted polystyrene. The grafting result was not totally specific since the grafted PS showed to be oxidized with again the oxygen only singly bonded to carbon probably due to air impurity in the process or to post oxidation of residual radical sites after venting the reactor.

In this paper, we report on first results on the assessment of biocompatibility of styrene and CE grafted HDPE by hepatocyte cultures. Hepatocytes were chosen because they are very demanding to the substratum in that anchoring to it is vital for their survival. On the other hand, hepatocytes offer distinct metabolic performance that can be taken as a measure of their vitality.

EXPERIMENTAL

HDPE (BASF "Lupolen 4261 A", thickness : 0.6 mm, with some non specified antioxidant) and polystyrene (BASF 2710 white 744) sheets were plasma grafted with styrene (ST) and (2-chloroethyl)vinyl ether (CE) as described in [1]. After 1 year of storage time, the samples were seeded with hepatocytes as described below. For comparison, one HDPE sample treated with CE vapour only (without any plasma) and an untreated HDPE and polystyrene (PS) sample were also tested. As a control and reference commercial tissue culture (TC) plastic (= polystyrene, TC quality supplied by Greiner) was run with the same tests.

Hepatocytes were isolated from the livers of male Sprague-Dawley rats (240-270 g) and cultured in serum-free Williams medium E as described [12] except that no coating with collagen was performed. Cells were seeded at a density of $1.25 \cdot 10^5$ cells/ cm² in the presence of 5 % newborn calf serum. The first change to serum-free medium was made after 3 h. Non-adhering hepatocytes were carefully removed at this time.

In order to assess adherence, spreading and maintenance of hepatocytes on the supporting material to be tested, small pieces of the test material were trimmed down to accurately fit into the wells of a 24-well culture tray (Costar). Before placing them on the bottom of the wells they were rinsed with sterile Hank's buffer pH 7.4. Cells were inoculated directly on the test material or the normal tissue culture (TC) surface of the wells (for control) using 0.5 ml/well.

Cells were stained with Trypan blue [13], carefully examined and stained subsequently with Eosine. Afterwards, measurements of the area occupied by the hepatocytes were performed using the image analyzer BAC 8810 as described [14]. Protein content was determined according to Lowry et al. [15]. Leakage of LDH (lactate dehydrogenase) through the cell membrane as well as production of urea was determined from the medium taken after 3 h (culture washed afterwards) and after 20 h as described previously [16, 17].

RESULTS

Table 1: Adherence, viability and metabolic performance of hepatocytes cultured on various plastic supports^{a), b)}

Material	Area covered by cells ^{c)} (% control)	Protein content		LDH leakage (mU/ml)		Trypan blue staining cells (%)	Urea (µmoles/mg)
		µg/cm ²	%	2 h	24 h		
Control (TC plastic)	100	153	100	307	64	2	1.7
PS, untreated	95	142	93	411	105	2	1.6
PS- CE plasma grafted	73	99	65	834	332	24	0.6
HDPE, untreated	81	127	83	489	122	11	1.2
HDPE, CE vapour only	78	121	80	527	109	10	1.3
HDPE-ST plasma grftd.	144	190	124	243	72	2	2.1
HDPE-CE plasma grftd.	71	105	69	788	252	15	0.9

a) Data were determined after 20 h except stated otherwise.

- b) Data were expressed as means of duplicate determinations, the maximum difference between measured values was below 15 %.
- c) The area covered by hepatocytes was expressed as percentage of the controls

On HDPE-derived supporting material adherence and spreading was checked by phase-contrast microscopy. Compared to controls on TC plastic adherence on styrene grafted HDPE (HDPE-ST) was almost as rapid, while on CE grafted (HDPE-CE) and untreated HDPE it was much slower. On these latter materials spreading was retarded resulting in a round shape of the cells even after 20 h of cultivation, whereas on HDPE-ST spreading was even better than on the TC plastic control. Unfortunately, both phenomena could not be evaluated on polystyrene based material because of its lacking transparency.

Trypan blue staining that marks the nuclei of fatally damaged cells indicates HDPE to be a less suitable substratum (see Table 1). CE grafting especially onto PS also leads to high losses of living cells.

The *area covered* by living cells (Fig. 1) is significantly the largest for the styrene grafted HDPE indicating that this material is an even better support than TC culture plastic. In contrast, the smallest covered areas were found on the CE grafted substrates that obviously have some rather repulsive effect on the hepatocytes. This goes perfectly parallel with the measured *total protein* content of the cells reflecting their number and viability.

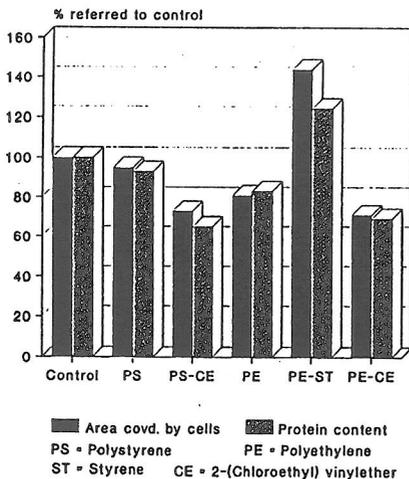


Fig. 1: Spreading and protein content of hepatocytes on different substrates

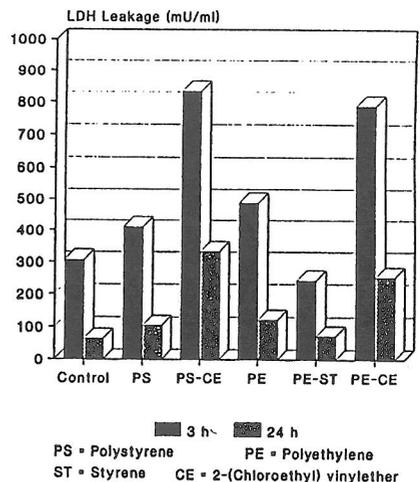


Fig. 2: LDH leakage from hepatocytes cultivated on different substrates

Evaluation of *LDH-leakage* from damaged cells (Fig. 2) after 3 h revealed that modifying the surface of HDPE or PS by CE grafting led to a supporting material of low quality. In contrast, grafting of styrene onto HDPE created a very good support equivalent to TC plastic. Similar results were obtained when *LDH-leakage* was measured after 20 h of cultivation.

Urea production is a strongly energy consuming process requiring the function of different compartments of the cells and hence, a good measure for their activity and metabolic performance. On the styrene grafted HDPE the highest and on both CE grafted materials the lowest urea production was found (Fig. 3).

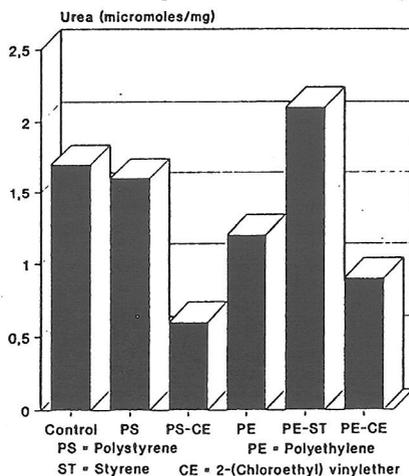


Fig. 3: Urea production of hepatocytes cultivated on different substrates

CONCLUSION

Several modified and unmodified plastic surfaces were compared as substrates for the cultivation of rat hepatocytes. Pure polystyrene surfaces of TC quality as provided by commercial products are sufficient for the adherence and maintenance of hepatocytes, but can be considerably improved by coating with collagen (12,13,17). In the present study, HDPE was found to be less efficient than polystyrene. Its supportive capacity, however, could be considerably improved by modifying its surface with styrene, while modification with CE diminished this capacity with HDPE and PS as base materials.

Since also the degree of oxidation is similar for styrene and CE grafted HDPE, it must be assumed that the chemical nature of the monomer to be grafted seems to be more important than the oxidation induced by the plasma.

It can be concluded that HDPE-ST might be superior to normal TC plastic surfaces for the cultivation of hepatocytes. Whether its supportive capacity can be further improved by coating with collagen remains to be investigated.

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