Porous Polymers by Emulsion Templating

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Summary: Highly porous and permeable polymers are produced by polymerisation of the continuous phase of high internal phase emulsions (HIPEs). The morphology and properties of the resulting PolyHIPE materials can be varied, allowing the materials to be optimised for a variety of applications. Void diameter is controlled from 1 to around 100 μm by altering the HIPE stability. Surface areas greater than 700 m^2g^{-1} can be achieved by replacing some of the monomer phase with non-polymerisable solvent, in conjunction with a high crosslink density and the use of a surfactant mixture that limits Ostwald ripening. PolyHIPEs can be produced in a variety of physical forms including large monolithic slabs, rods and flat relatively thin membranes. The materials are currently under investigation for use as electrochemical sensor membrane substrates and as porous matrices for cell culture.

Keywords: cell culture; emulsion; foams; PolyHIPE; sensors

Introduction

Highly porous polymer foams are attractive materials for a wide range of advanced applications, including as matrices for cell culture and tissue engineering, supports for solid phase synthesis and species employed in solution phase synthesis (reagents, catalysts and scavengers), separation media and as sensor substrates. Many different types of polymer foam are available, however these are usually prepared by gas blowing techniques and tend to have large cell sizes, irregular morphologies and can be closed cell. It is likely that advanced materials applications will require well-defined porous materials with tuneable morphologies and properties. One method of foam production that offers such a level of control is the use of high internal phase emulsions (HIPEs, which are emulsions with a droplet phase volume ratio above 0.74[1]) as templates for porous material preparation. To achieve this, one or more monomers plus, usually, a crosslinker, is present in the continuous, or non-droplet, phase of the emulsion. Curing of the monomer(s)

 DOI: 10.1002/masy.200550819

occurs *around* the dispersed phase droplets, which, when removed post-cure, create the voids in the resulting highly porous material (known as a PolyHIPE).[2] Volume contraction on conversion of monomer to polymer ensures that each void is connected to all of its neighbours by 'windows'.[3] The result is an open-cell solid foam with an extremely well-defined morphology (Figure 1). Of vital importance to the use of PolyHIPEs in advanced materials applications is the ability to control morphology (surface porosity, void/interconnect size) and physical properties (surface area). In this paper, we describe some of our recent results on controlling various aspects of PolyHIPE morphology and properties, facilitating the development of novel materials as sensor supports and as matrices for cell growth.

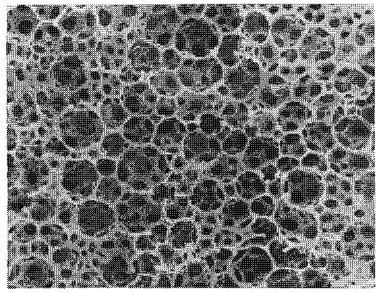

Figure 1. SEM of PolyHIPE material.

Experimental

Materials. Styrene, divinylbenzene (DVB) and 2-ethylhexyl acrylate (EHA) were freed of inhibitor by passing through a short column of basic alumina. All other chemicals were used as received.

Methods. Poly(styrene-DVB) and poly(styrene-EHA-DVB) PolyHIPEs (aqueous:organic phase ratio = 9:1) were prepared according to a procedure described elsewhere,[4] employing either Span 80 or a mixture of Span 20, dodecylbenzenesulfonic acid, sodium salt (DDBSS) and cetyltrimethylammonium bromide (CTAB)[5] as the surfactant. To prepare electrochemical sensor materials, a piece of PolyHIPE membrane was immersed in a solution of potassium ionophore I (valinomycin), plasticizer (bis(2-ethylhexyl)sebacate, BEHS) and lipophilic anionic additive (potassium tetrakis(4-chlorophenyl)borate, PTCB) in tetrahydrofuran in a glass beaker (PolyHIPE:BEHS:valinomycin:PTCB ratio = 33:64.7:1.3:0.6 (w/w)). The solvent was allowed to evaporate very slowly, and the active

agents were adsorbed by the PolyHIPE membrane matrix/pore wall. Potassium ion electrodes were constructed by assembling the PolyHIPE potassium selective membranes in a Philips IS-561 electrode body. The electromotive force (EMF) of the electrodes in a set of aqueous KCl solutions was measured at room temperature with a Molspin pH4 meter in AC mode. A Unicam RE15 electrode was used as the reference electrode. Addition of 1% (v/v) THF to the aqueous phase was used to increase the void size to prepare materials for cell culture. PolyHIPE scaffolds were subsequently coated with poly(D-lysine) (10 μg/ml) followed by human placental laminin (10 μg/ml) by submerging the polymers in the appropriate aqueous solution overnight before being washed (three times) in phosphate buffered saline (PBS). Aggregates of neural cells derived from human TERA2.cl.SP12 embryonal carcinoma stem cells were collected and seeded onto matrixes maintained in DMEMFG and cultured for 7 days. After the growth period, the 3-D cultures were washed in PBS (two times) and fixed at 4 °C for 90 min in a solution of 2.5% (w/v) glutaraldehyde, 1% (w/v) paraformaldehyde, 0.01M NaOH, and 0.1M Sorensen's phosphate buffer (pH 7.2). Fixed cells were then stained at 4 °C for a further 60 min using 1% (w/v) osmium tetroxide in phosphate buffer (pH 7.2). Samples were dehydrated using a graded series of ethanol solutions and dried using CO_2 critical point drying.

Analytical techniques. Scanning electron microscopy (SEM) was performed with a Philips/FEI XL30 ESEM, either on uncoated samples employing the back-scattered electron detector, or on samples sputter-coated in gold (Edwards S150B sputter coater) using the secondary electron detector. Surface areas were determined by applying the BET model[6] to nitrogen adsorption data obtained with a Micromeritics Tristar 3000 gas adsorption analyzer.

Results and Discussion

High surface area PolyHIPE materials could potentially find application in areas such as heterogeneous catalysis, solid phase extraction and liquid chromatography. Surface areas of poly(DVB) PolyHIPEs can be increased by two orders of magnitude, up to 350 m^2g^{-1} by replacing some of the monomer in the HIPE continuous phase with a nonpolymerizable organic solvent, such as toluene.[7] However, the resulting materials are extremely weak mechanically. Using a solvent that is more compatible with the growing polymer network, such as 1,2-dichlorobenzene, results in a material with a surface area of around 500 m^2g^{-1} (Table 1). However, even greater surface areas, in excess of 700 m^2g^{-1}, were obtained

when the surfactant was changed from Span 80 to a mixture of nonionic (Span 20), cationic (CTAB) and anionic (DDBSS) surfactants.[8] Furthermore, the materials were significantly more robust than those prepared with Span 80 plus organic porogenic solvent, and could be handled easily. The surfactant mixture is known to form a strong interfacial film, which could inhibit Ostwald ripening (a process whereby large emulsion droplets grow at the expense of small ones, due to migration of droplet phase molecules through the continuous phase). Ostwald ripening will lead to the presence of significant quantities of water in the continuous phase, making it a poorer solvating medium for the growing polymer network. By limiting this process, the surfactant mixture leads to materials with higher surface areas. Pulsed-gradient spin echo NMR experiments were used to determine the self-diffusion coefficient of water, *D*, in emulsions containing the two surfactants. Those prepared with the surfactant mixture resulted in values of *D* of around 2.5 x 10^{-10} m^2s^{-1}, whereas Span 80 resulted in *D* values of around 7.0 x 10^{-10} m^2s^{-1}.

Table 1. Surface area values of polyDVB PolyHIPEs prepared with oil-phase soluble porogenic solvents.

Solvent[a]	**Surfactant**[b]	**Surface Area/m^2g^{-1}**
Toluene	Span 80	360
1,2-Dichlorobenzene	Span 80	500
Toluene	Mixture	720
Chlorobenzene	Mixture	690

a – 1:1 (v/v) relative to DVB.
b – Span 80: 20% (w/w) relative to DVB; mixture: 7% (w/w) relative to DVB. Composition of mixture defined in the text.

Another parameter that is important to be able to control is the surface porosity of the materials. Articles prepared by moulding processes should possess fully open surfaces to allow the unrestricted ingress of fluids. Glass as a mould substrate caused adhesion of poly(styrene/DVB) materials to its surface and the PolyHIPE surface in contact with the glass had a different morphology from the fractured surface. Poly(vinyl chloride) (PVC) was found to compromise HIPE stability, possibly due to leaching of plasticizer, and any PolyHIPEs that formed tended to adhere to the substrate. PolyHIPE prepared against polypropylene (PP) had a non-porous skin on the surfaces in contact with PP, presumably

from a phase separated monomer film at the substrate-HIPE interface. In contrast, poly(tetrafluoroethylene) (PTFE) did not result in surface bonding and produced an open and porous surface structure (Figure 2). Previously, Akay et al.[9] found that polyethylene and PTFE produced a closed-cell PolyHIPE surface, PVC resulted in surface bonding and glass gave rise to an open-cell structure. They ascribed these effects to differences in solubility parameter ($\Delta\delta$) between substrate and monomer. The reasons for the different performances in our hands could be due to variations in HIPE composition or preparation procedure.

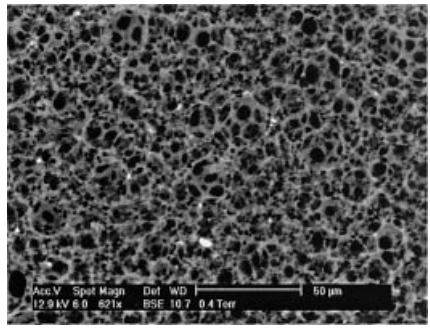

Figure 2. SEM of the surface of a PolyHIPE membrane in contact with the PTFE mould substrate.

Such materials were used to prepare porous electrochemical sensors, which integrate separation (of e.g. dust and soil particles, blood cells, etc.) with sensing. To achieve this, PolyHIPE membranes of thickness of around 100 µm were required. These were prepared in flat moulds, consisting of two PTFE plates separated by poly(ethylene terephthalate) (PET) rings of specified thickness. Membranes of the correct diameter were obtained from the master membrane by a simple stamping process. It was found that PolyHIPE membranes so prepared were prone to localized emulsion collapse on the surfaces in contact with the mould substrate. This resulted in the formation of pin-holes and other defects. Careful optimisation of the emulsion formulation ([Span 80] = 24% (w/w) relative to monomer concentration; aqueous/organic phase ratio = 8:2) allowed the preparation of glassy poly(styrene-DVB) and elastomeric poly(styrene-EHA-DVB)[10] membranes with large (> 6 cm^2) pin-hole free areas and sufficient mechanical strength to permit easy handling.

The thin PolyHIPE membranes were used to prepare porous potentiometric sensors (ion selective electrodes). PolyHIPE membranes were swollen in THF solutions of potassium ionophore I, a lipophilic anionic additive (PTCB) and a plasticiser (BEHS). Slow evaporation of the solvent resulted in a weight gain of around 200% and an area increase of around 40%. The electromotive force (EMF) responses of the membranes to K^+ were measured with a potentiometer and their performance was compared to that of conventional non-porous PVC membranes. The PolyHIPE membrane gives a linear response (slope = 52-58 mV) to a lower concentration (Figure 3), which translates into a lower level of detection (down to 10^{-5} M) compared to PVC membranes. Selectivity for K^+ over other cations was 10 times better than for the PVC membrane, and the response times are quicker (always less than 60s) at low analyte activities.

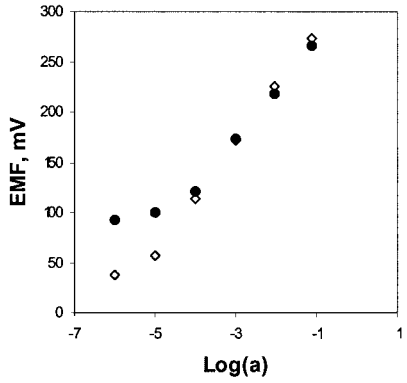

Figure 3. Potentiometric response of K^+ ion selective membranes: PVC (•); elastomeric PolyHIPE (◊) (HIPE organic/aqueous phase ratio=2:8, St:EHA:DVB=30:60:10).

In addition to surface area and foam outer surface porosity, another parameter that must be controlled in order to prepare tailored materials is average void diameter. Typically, void diameters in the region of 10 μm are observed in 90% porous poly(styrene-DVB) PolyHIPEs.[11] While a small diameter is required for certain applications, such as liquid chromatography and heterogeneous catalysis, other uses necessitate much larger void sizes. One such application is cell culturing, where the diameters of the voids and, more critically, the windows, must be sufficiently large to allow migration of cells throughout

the matrix. The minimum void size required depends on the cell type to be cultured, but as a rough guide the average void diameter should be greater than 20 μm. Obviously, PolyHIPE materials prepared in the usual manner do not posses the correct void dimensions. It is known that void size is inversely correlated with emulsion stability, therefore to increase void diameter our strategy should be to *destabilize* the emulsion (but in a controlled manner, as emulsion phase separation before the onset of cure may occur). During our work on increasing the surface area,[8, 12] we noticed that higher surface areas were accompanied by smaller average void diameters. Therefore, promoting Ostwald ripening could be a useful approach to create larger emulsion droplets and therefore a higher average void diameter. Small quantities of water-miscible organic species (tetrahydrofuran (THF), methanol, poly(ethyleneoxide) (PEO)) were added to the aqueous phase of styrene-DVB HIPEs ([Span 80] = 20% (w/w); aqueous/organic ratio 9:1), which were then cured. It was found that 1% (v/v) THF in the aqueous phase produced a material with a much greater void diameter than the control (no THF) (Figure 4). The mechanical strength of these foams was similar (qualitatively) to those prepared without THF.

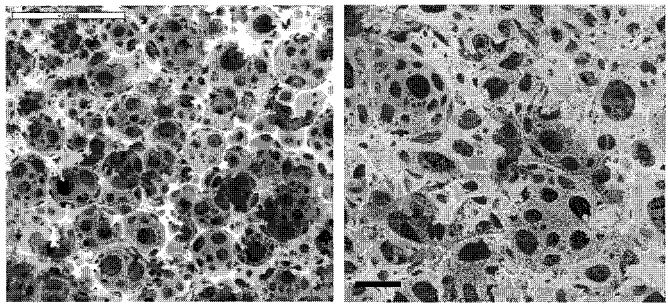

Figure 4. SEMs of Poly(styrene-DVB) PolyHIPEs without (left) and with 1% (v/v) THF added to the HIPE aqueous phase (scale bar on right = 50μm).

The resulting materials were coated in poly(D-lysine) and/or laminin and used as substrates for the culture of human neurons derived from embryonic carcinoma stem cells. Aggregates of cells were seeded onto discs of PolyHIPE and cultured for 7 days. Conventional culturing in 2D (tissue culture plastic) was also conducted as a control. After 7 days, it was found that the cells adhered readily onto the coated PolyHIPE material

and individual cells and small aggregates were seen in the material interior (Figure 5). Furthermore, cells cultured in 3D (PolyHIPE) were observed to have formed much more extensive networks of individual neural processes than those grown in 2D. Protein analysis from cell lysates indicated that markers of maturation of the 3D neural network (MAP2a,b) were up-regulated in neurons cultured on PolyHIPE.[13] This indicates that the environment in which the cells are cultured (2D vs. 3D) influences significantly their development.

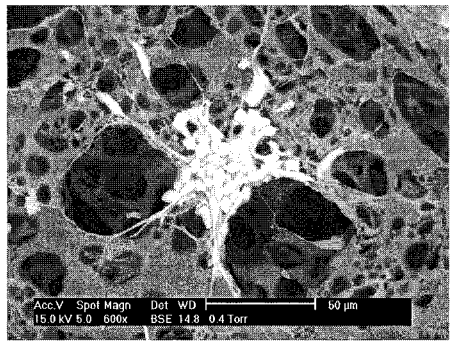

Figure 5. An aggregate of neurons adhering to coated PolyHIPE.

Conclusion

Control of the morphology and physical properties of PolyHIPEs is key to their development for advanced materials applications. By careful optimization of the composition and processing conditions of the emulsion precursor, we are able to produce homogeneous materials with tailored surface areas and void diameters. These materials are proving advantageous in applications such as sensor technology and cell culturing.

Acknowledgements

The authors thank the following organisations for funding: Engineering and Physcial Sciences Research Council (EPSRC) for AB (GR/M02194) and CZ (GR/83064), Biotechnology and Biological Sciences Research Council (BBSRC) for MH (12/G16231) and Qinetiq for RJC.

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