Poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) as a Mouse Embryonic Stem Cells Support

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ABSTRACT: A series of swellable ethylene dimethacrylate-crosslinked poly(2-hydroxyethyl methacrylate) (PHEMA) sheets of homogeneous (nonporous) structure or with different degrees of swelling and porosities was produced by bulk polymerization in either the absence or the presence of various diluents (porogens). Calculations performed by use of the solubility parameter δ of the reaction components indicate that the solvation conditions of the polymerization system change, depending on the solvating power of the diluent, which thus controls the porosity. Pore volume also seemed to be sensitive to the presence of the linear polymer diluent. Polystyrene (PS) showed, compared with poly(methyl methacrylate) (PMMA), a higher precipitating ability to form porous PHEMA sheets with an increased pore size because of its higher noncompatibility with newly formed crosslinked PHEMA. Given that PHEMA hydrogel is well known for its biocompatibility, it was used here as a potential carrier of cells in transplantation therapies. Attachment and growth of mouse embryonic stem (ES) cells on gelatin-coated transparent PHEMA hydrogel substrates were examined. Two days after plating, survival and morphology of ES cells were largely similar on both PHEMA hydrogel sheets and in petri dishes as controls. This suggests that PHEMA hydrogels are likely candidates for application in transplantation therapies involving ES cells. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 87: 425–432, 2003

Key words: poly(2-hydroxyethyl methacrylate); porosity; embryonic stem cells; hydrogels; crosslinking

INTRODUCTION

Poly(2-hydroxyethyl methacrylate) (PHEMA) hydrogel has attracted attention as a useful material in a wide variety of medical and biological applications, such as soft contact and intraocular lenses, implants, wound dressing, drug delivery systems, membrane chromatography, and carriers for immobilization of enzymes, antibodies, and cells.[1] It is nontoxic, biocompatible, swells but does not dissolve in aqueous media, and meets nutritional and biological needs of the cells. Its probability of being rejected by the body is low because there is low interfacial tension between the swollen gel surface and aqueous environment, which minimizes protein interaction with the implant. Its high compliance can also reduce frictional irritation of surrounding tissues.[2] PHEMA is essentially a nonadherent material for the cells; therefore it has to be preincubated with gelatin if used for cell cultivation. Hydroxy groups are available for further modifications to promote cell attachment, proliferation, differentiation, and migration.

Recently, there has been a growing interest in scaffolds for tissue and organ reconstruction and substitution.[3] The scaffolds function as substrates for proliferation and differentiation of cells seeded or infiltrated from the surrounding host tissue, which are finally integrated in the regenerated tissue, thus restoring the organ function. Cell–scaffold interaction is greatly influenced by their porous structures, especially by the pore size, important factors in organ regeneration. Depending on the type of tissue or cells, there is an optimum pore size and surface area required for cell infiltration, their attachment, and subsequent host tissue ingrowth. For example, the fibrovascular tissue ingrowth rate into poly(vinyl alcohol) sponge sheets showed a maximum at pore sizes around 250 μm as a result of the balance of channel size for cell infiltration and their surface area for cell attachment.[4] PHEMA sponges used in keratoprosthesis had to have at least 10- to 20-μm pores to promote incorporation of the host tissue into the prosthetic skirt through cellular invasion and growth.[5] Porosity can be introduced into

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PHEMA by a number of methods, which include polymerization around a crystalline matrix (ice, D-glucose, saccharose, NaCl) that is subsequently dissolved to leave pores,[6] photolithography, or other microfabrication technologies.[2]

Another technique induces phase separation between the polymer and diluent, which can be its thermodynamically "good" (ethanol, glycerol, ethylene glycol, cyclohexanol) or "bad" solvent (diacetin, dodecan-1-ol), or a polymer or a mixture of various diluents, including aqueous NaCl solution. In addition to porosity, pore structure and surface area, other parameters, which are considered to describe overall performance of the material in applications, include swellability, specific functional sites, and the shape of the device. The polymer scaffold can be molded into the desired shape, such as spherical particles, monoliths, tubes, discs, slabs, or membranes, the last mentioned form being the most popular. Several developed membrane (sheet) fabrication techniques include film-casting and particulate-leaching,[7] photolithography, and molding polymerization.[2]

The aim of this study was to induce phase separation by means of both low and high molecular weight organic diluents and to prepare both low- and high-crosslinked PHEMA sheets differing in porosity, water content, and surface area. Because these sheets, both homogeneous and with an increased pore size, are intended, after various pretreatments, to provide scaffolds for cell therapy, the second objective of the present study was to investigate whether these matrices may serve as carriers of embryonic stem (ES) cells.

EXPERIMENTAL

Reagents and cells

2-Hydroxyethyl methacrylate (HEMA), supplied from Röhm GmbH (Germany), was purified by distillation; ethylene dimethacrylate (EDMA) was purchased from Ugilor S.A. (France) and purified by the same method. 2,2′-Azobisisobutyronitrile (AIBN; Fluka Chemie, Buchs, Switzerland) was crystallized from ethanol and used as initiator. Cyclohexanol (Lachema, Czech Republic) was distilled; other solvents were obtained from Aldrich (Steinheim, Germany) and used without further purification. Krasten 127 polystyrene (PS; M_w = 240,000; M_n = 90,000) was obtained from Kaučuk Kralupy (Czech Republic), and poly(methyl methacrylate) (PMMA; M_w = 150,000) obtained from Považské chemické závody (Žilina, Slovakia). The J1 line of ES cells was kindly provided by Dr. Chu-xia Deng (National Institutes of Health, Bethesda, MD). Gelatin from porcine skin, cell culture tested, approximately 300 bloom, and all other chemicals were obtained from Sigma (St. Louis, MO).

Sheet preparation

The PHEMA sheets (3 mm thick) were prepared by bulk polymerization at 70°C for 10 h between two Teflon sheets (10 × 10 cm) separated by a silicone rubber gasket. The porogen (diluent) level, expressed as a volume percentage of the whole polymerization mixture, was 60 vol %; polymeric porogen (PS or PMMA) was expressed as a weight fraction of the porogen. The polymerization mixture (15 mL) was flushed by bubbling nitrogen for 10 min and injected into a preheated mold. The resulting products prepared in the presence of a low molecular weight porogen were washed with water, water/ethanol (90/10, 60/40, 30/70 v/v) mixtures, and ethanol to remove the diluent, unreacted monomers, and initiator residues. Ethanol was then stepwise replaced again with water, in which sheets were finally kept. If PMMA or PS was used as a high molecular weight porogen in the synthesis, an extensive washing with acetone or toluene, respectively, preceded the previous procedure.

Equilibrium water content

All sponge sheet samples (1 × 2 cm in size) were kept for 2 weeks in deionized water, which was exchanged daily. The samples were blotted with filtration paper, weighed in their fully hydrated state, and then dried in an oven (50°C) for 2 days. The dehydrated samples were weighed again. The equilibrium water content (EWC), g (mL) of water per g of dry polymer, was calculated by the following equation:

$$\mathrm{EWC} = (w_w - w_d)/w_d$$

where w_w and w_d are the weight of a hydrated specimen and the same specimen after drying, respectively. The results are average values of five measurements for each sheet.

Porous properties

To measure pore volume, the sheets, which were first equilibrium swollen in water, were washed with acetone and then with cyclohexane. Through the use of this procedure, called solvent-exchange, the good (swelling) solvent in the swollen gel was replaced with the nonsolvent. Cyclohexane regain was measured by a centrifugation method, as described in the literature.[8] Specific surface area was measured by dynamic desorption of nitrogen (Quantasorb; Quantachrome, Greenvale, NY), the average pore radius was calculated from the equation $r = 2000(V/S_{\mathrm{BET}})$ (nm), where V is the pore volume from cyclohexane regain and S_{BET} is the specific surface area, and a cylindrical model was assumed for the pore shape.[9]

The morphology of sample cross sections after critical-point drying (Balzers Union, Balzers, FL) was investigated by use of scanning electron microscopy (SEM; JSM 6400; JEOL, Peabody, MA) after surface coating with a 4-mm Pt layer (vacuum sputter coater SCD 050; Balzers).

Cell culture

J1 ES cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco/BRL, Gaithersburg, MD) supplemented with 20% fetal calf serum, 2 m*M* glutamine, 0.1 m*M* nonessential amino acids, 0.05 m*M* 2-sulfonylethan-1-ol, 100 IU/mL penicillin, 0.1 mg/mL streptomycin, and 2×10^3 U/mL leukemia inhibitory factor (LIF; Gibco/BRL). To examine the PHEMA hydrogel sheet as a substrate, the cells were seeded at a density of 8×10^4 per mL on a 96-well tissue-culture plate containing PHEMA hydrogel fragments preincubated with 0.1 wt % gelatin in distilled water for 24 h. Control ES cells were seeded on gelatinized 96-well tissue-culture plates. The cells were grown for 48 h and photographed with an Olympus IX70 microscope (Olympus, Lake Success, NY) equipped with a Hoffman contrast. In a parallel experiment we determined the amount of cells growing on gelatinized PHEMA fragments and on a tissue-culture plastic by fluorimetric measurement of DNA concentration in cell extracts. DNA quantification by fluorimetry was performed by use of DNA-bound fluorochrome Hoechst 33258 (Hoechst, Frankfurt/Main, Germany) excited with UV light at 365 nm and emitting at 458 nm. Quantitative data were derived from at least eight measurements and are presented as arbitrary units.

RESULTS AND DISCUSSION

PHEMA sheets were prepared by the bulk polymerization technique in a specially designed mold with Teflon-covered plates. In preliminary studies it was observed that variation of the initiator concentration does not alter physical and chemical properties of the polymer. Therefore, keeping this parameter constant at 1 wt % relative to the monomers, and also a constant initial volume fraction of the monomers in the polymerization mixture at 0.4 throughout, only the type of diluent (porogen) and EDMA concentration in the monomer phase (2 or 40 wt %) were varied. If a linear polymer was added to the diluent phase, its amount was changed. A porous structure was obtained by removing (washing) diluents after polymerization. Polymerization conditions and properties of PHEMA sheets are listed in Table I. The sheets can be divided into three groups. The copolymers in series I, prepared without a diluent, are called homogeneous (nonporous), in contrast to heterogeneous (porous) structures in series II and III, produced with a low and high molecular weight porogen, respectively. Sheets were transparent in series I compared with opaque or white copolymers in series II and III. PHEMA samples in series III were characterized by a weak mechanical strength and they were quite friable if prepared with high EDMA contents. Given that all the samples possessed specific surface areas less than 25 m^2/g, it can be assumed that no micro- (diameter < 2 nm) or mesopores (2–50 nm in diameter) were present in the structure.

The porous structure characterization involved determination of specific surface area, SEM of sheet cross sections after critical-point drying from ethanol, and solvent uptakes through use of solvents with different affinities to the polymer chain: water and cyclohexane [i.e., good (swelling) and bad (precipitant) solvent, respectively]. Although the copolymers showed a substantially increased swelling in ethanol (the best solvent for PHEMA) compared with that in water, characterization in water is important because all prospective tissue engineering applications are performed in aqueous media. It is evident from Table I that PHEMA systematically imbibed more water (up to 2 mL/g) than cyclohexane. Water uptakes of macroporous copolymers can be considered a result of two contributions: filling of the pores and their swelling by chain solvation. The uptake of cyclohexane, which cannot swell the copolymer, is the result of the first contribution only. Therefore it reflects the pore volume. The cyclohexane regain is known to give pore volume values very similar to mercury porosimetry results.[10] The fact that cyclohexane uptakes were lower than EWC demonstrates that swelling of the inner structure has occurred in water. The pore volume data in connection with specific surface area enable determination of the average pore radius, which does not necessarily correspond to the size obtained by mercury porosimetry. The information potential of this value, which was calculated by use of a cylindrical model for the pore shape, is rather limited in the range of low porosities and surfaces, which are determined with insufficient accuracy, and therefore such data are omitted in Table I.

Formation of porous structure

As stated erlier, the copolymers prepared without addition of diluent to the polymerization system possessed homogeneous structures [i.e., their surface area and cyclohexane regain were negligible (series I in Table I)]. In the presence of an inert diluent as a pore-forming agent (porogen) in a polymerization mixture, the formation of heterogeneous (macroporous) structures may result if the precipitation process (phase separation) between the diluent and polymer phase occurs during the crosslinking copolymer-

TABLE I
PHEMA Sheet Synthesis Conditions and Properties

Sheet/series	Crosslinking (wt %)	Low/MW diluent[a]	High MW diluent/weight fraction[b]	Spec. surface area (m^2/g)	EWC[c] (mL/g)	Cyclohexane regain (mL/g)	Pore radius (nm)	δ_1^d ($MPa^{1/2}$)	$(\delta_1 - \delta_2^e)^2$ (MPa)
1/I	2			0.12	0.60	0.04	—[f]	23.2	42.2
2/I	40			0.04	1.87	0.05	—[f]	21.4	68.9
1/II	2	COH		0.16	0.66	0.05	—[f]	23.3	41.0
2/II	2	COH/DOH[g]		1.27	1.38	0.77	1212	22.4	53.3
3/II	2	DOH		1.48	1.47	1.03	1392	21.3	70.6
4/II	2	DCHE		0.1	1.30	1.27	na	21.1	74.0
5/II	40	COH		25.6	1.87	1.65	129	22.6	50.4
6/II	40	COH/DOH[g]		0.09	1.29	1.16	na	21.8	62.4
7/II	40	DOH		5.00	1.81	1.06	424	20.6	82.8
8/II	40	DCHE		21.9	1.66	1.69	154	20.4	86.5
1/III	2	DCHE	PMMA/0.1	0.12	1.21	1.23	na	20.9	77.4
2/III	2	DCHE	PMMA/0.2	0.37	1.49	1.27	6865	20.8	79.2
3/III	2	DCHE	PS/0.1	0.08	1.43	1.40	na	20.9	77.4
4/III	2	DCHE	PS/0.2	0.11	1.80	1.72	na	20.7	81.0
5/III	40	DCHE	PMMA/0.1	3.94	1.22	0.75	361	20.3	88.4
6/III	40	DCHE	PMMA/0.2	13.7	1.92	2.00	311	20.1	92.2
7/III	40	DCHE	PS/0.1	0.45	1.43	0.79	3511	20.2	90.2
8/III	40	DCHE	PS/0.2	5.64	1.55	1.23	436	20.1	92.2
9/III	40	DCHE	PS/0.35	10.5	2.08	1.92	366	19.9	96.0

[a] The diluent concentration was 60 vol % of the whole polymerization mixture.
[b] Weight fraction of the whole diluent phase.
[c] Equilibrium water content.
[d] Solubility parameter of the starting polymerization mixture.
[e] Solubility parameter of the PHEMA.
[f] Homogeneous sample. COH, cyclohexanol; DOH, dodecan-1-ol; DCHE, 1,2-dichloroethane; PS, polystyrene; PMMA, poly(methyl methacrylate); na, nonapplicable.
[g] COH/DOH = 3/2 v/v.

ization.[11] The design of the pore structure (porosity, surface area, pore size) has been investigated in many polymer systems.[12] It has been shown that the structure depends on selection of the type of diluent chosen, which can be a solvent, a nonsolvent (precipitant), or a linear polymer. The porous structure of the resulting polymer product and its swelling in solvents vary with the distribution of the diluent between network and diluent phases (diluent in the pores) at the end of the polymerization, depending on the polymer/diluent interaction in a mixture. In other words, the porous structure is controlled by the solubility of the formed copolymers in the diluents.

When the inert diluent has a high solvating power (a thermodynamically good solvent for the polymer), two kinds of porous structures can be obtained: expanded or macroporous. The diluent may remain in the network (gel) phase throughout the polymerization at low degrees of crosslinking, resulting in the formation of an expanded (swollen), relatively homogeneous (nonporous) matrix. This was the case of cyclohexanol (solvating diluent for the PHEMA copolymer; see below) giving sheets of negligible surface area, which virtually did not retain any cyclohexane (very low pore volumes; sample 1/II in Table I). Cyclohexanol may also separate from the network phase at a high EDMA concentration in the polymerization mixture, resulting in porosity formation. This is exemplified by sample 5/II in Table I characterized by relatively high specific surface area, 25.6 m^2/g, and pore radius around 130 nm. On the other hand, polymerization in the presence of low-solvency diluent (a thermodynamically poor solvent for the polymer) leads to phase separation during the conversion of liquid monomers to solid polymers and to the formation of highly porous globular structures. Dodecan-1-ol and 1,2-dichloroethane are examples of such a solvent for PHEMA (see below). A wide range of porosities can be produced, depending not only on the nature and the percentage of diluent but also on the crosslinking degree.

A useful parameter that exerts some influence on characteristics of the copolymer pore structure formed is the diluent solvating power (solubility parameter). According to the Hildebrand theory, the solvating power of a polymer–diluent medium can be estimated from $(\delta_1 - \delta_2)^2$, where δ_1 and δ_2 are solubility parameters for the diluent and the polymer, respectively. Thus, the solubility of a polymer in a diluent is favored when $(\delta_1 - \delta_2)^2$ is minimized, that is, when the solubility parameters of the two components closely match. If there is an appropriate difference in δ value

TABLE II
Solubility Parameters δ of Components of the Polymerization Mixture[a]

Component	δ ($MPa^{1/2}$)
HEMA	23.3
EDMA	18.2
Cyclohexanol	23.3
Dodecan-1-ol	20.2
1,2-Dichloroethane	19.8
PMMA	19.0
PS	18.4

[a] Barton, 1991.[13]

between the diluent and the formed copolymer, the diluent acts as a precipitant of the copolymer and phase separation occurs during the polymerization process, and a macroporous copolymer sheet is formed. Because the final polymer forms from an initially homogeneous mixture of miscible liquids (monomers and diluents), all its components should be considered in calculation of the resulting solvating power. Averaging the solubility parameter of the components is given by

$$\delta = (\Sigma v_i \delta_i^2)^{1/2}$$

where v_i and δ_i are the volume fraction and solubility parameter of component i, respectively. Solubility parameters of the individual components[13] used in this study are listed in Table II. The last column in Table I contains the difference $(\delta_1 - \delta_2)$,[2] where δ_1 and δ_2 are solubility parameters of the starting polymerization mixture and PHEMA, respectively. The solubility parameter of lightly crosslinked PHEMA was taken as $\delta_2 = 29.7$ $MPa^{1/2}$ from the literature.[14] The difference between the solubility parameters of the starting polymerization mixture and of PHEMA shown in the last column of Table I indicates that both the monomer HEMA and cyclohexanol are good diluents for the polymer, so that they are solvating diluents for the system. On the other hand, dodecan-1-ol and 1,2-dichloroethane are bad diluents and should act as precipitants in the copolymerization. As a result, dodecan-1-ol and 1,2-dichloroethane should produce higher porosities than that of cyclohexanol. The solvating power of a diluent mixture depends not only on the affinity of each diluent to the copolymer but also on the interaction of diluent molecules.[15]

Low molecular weight diluent

To investigate the influence of a low molecular weight porogen on the porosity of PHEMA sheets, diluents used earlier in the preparation of macroporous supports by suspension polymerization[16] were included in the sheet copolymerization recipe. They included cyclohexanol (a good solvent for PHEMA), dodecan-1-ol, and 1,2-dichloroethane (both are poor solvents). The surface area, pore volume, and radius of porous PHEMA depended on variation of solubility parameters. A sheet with the lowest pore volume was obtained when neat cyclohexanol was used as a good inert diluent at 2 wt % of the crosslinking agent, indicating the formation of a gel-type structure. Samples prepared in the presence of poor solvents were already macroporous. In a system with a low content of crosslinking agent, the pore volume in the network increased as the solvating power of the diluent decreased, that is, with increasing difference between the solubility parameter of the polymerization mixture and PHEMA (Table I). The results clearly indicate different behaviors of cyclohexanol and dodecan-1-ol (1,2-dichloroethane) as pore-forming agents. Homogeneous structures resulted at $(\delta_1 - \delta_2)^2 < 50$ MPa, whereas macroporosity was formed at $(\delta_1 - \delta_2)^2 > 50$ MPa. A well-developed globular structure (globuli size ~ 2 μm) of such sheets obtained with dodecan-1-ol as an inert diluent is documented in Figure 1(a), (b).

When a mixture of cyclohexanol and dodecan-1-ol was used, which combines the properties of solvating (good) and nonsolvating (bad) diluent, a porous structure resulted with intermediate characteristics compared with those obtained with pure diluents. At the same time, the average pore diameter of all PHEMA sheets prepared at low degrees of crosslinking was greater than 1000 nm. These results can be attributed to the occurrence of a more pronounced phase separation caused by an increase in the nonsolvating diluent content (dodecan-1-ol, 1,2-dichloroethane). High amounts of crosslinking agent (40 wt %) in the polymerization mixture produced structures with pore volumes greater than those in the previous case and pore radii in the range of hundreds of nm; however, no significant dependency on the solubility parameter values was found. The highest values of the specific surface area (almost 26 and 22 m^2/g) were obtained when either cyclohexanol or 1,2-dichloroethane was used as the diluent, respectively.

High molecular weight porogen

In contrast to low molecular weight porogens, linear polymers contribute to the porogenic potency not only as a function of their concentration in the system but also through contributions related to their high molecular weight and composition.[17] To study the effect of polymeric porogen on swelling behavior, polystyrene and poly(methyl methacrylate) were chosen. Because both polymers are insoluble in HEMA, they were first dissolved in 1,2-dichloroethane and the solution was added to the polymerization mixture. The results show a great effect of the polymeric porogen.

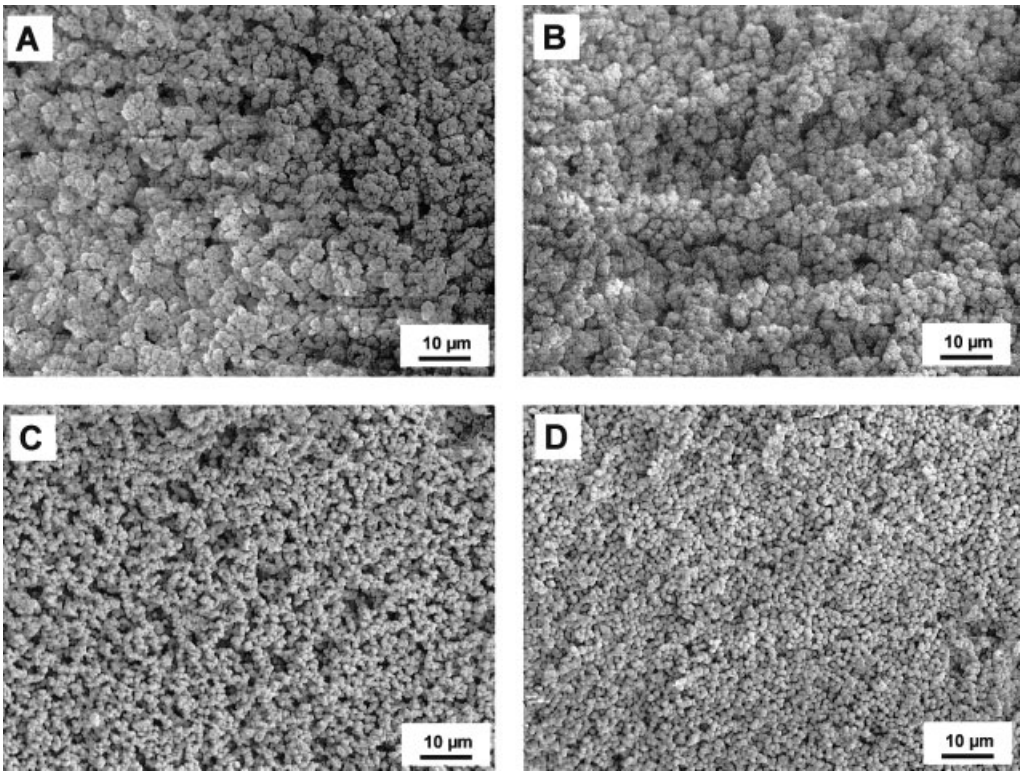

Figure 1 SEM micrograph of a cross section of sample 3/II (A), 7/II (B), 4/III (C), and 8/III (D). For sample composition, see Table I.

The resulting sheets were very fragile and with large holes in the structure. The largest pore volume, 2 mL/g, was obtained at 40 wt % of EDMA when a 20 wt % solution of PMMA in 1,2-dichloroethane was used as an inert diluent. PHEMA sheets formed in a 1,2-dichloroethane solution of a linear polymer as inert diluent exhibited, compared with the copolymers prepared in a mixture of cyclohexanol and dodecan-1-ol, larger average pore sizes. Samples with low EDMA contents had the largest average pore radii in the thousand nm range. From SEM of samples after critical-point drying [Fig. 1(c), (d)] it is quite obvious that interglobular distances (pores) in sample 4/III (2 wt % EDMA) are substantially larger than those in 8/III (40 wt % EDMA). The size of globuli in both samples is around 1 μm. However, whereas the largest pores in sample 4/III reach up to 10 μm [Fig. 1(c)], pores in sample 8/III show only 0.5 μm [Fig. 1(d)]. This is in qualitative agreement with the pore radius data calculated from specific surface areas and cyclohexane regain (Table I).

To obtain PHEMA sheets with different porous characteristics, the relative amount of polymeric porogen in 1,2-dichloroethane was changed (Table I). The swellability in water (EWC), pore volume, and surface area increased when the concentration of high molecular weight porogen was increased. An increase in both the PS and PMMA concentration in 1,2-dichloroethane leads at the same time to samples with smaller pore radii. It is interesting to compare PS and PMMA as diluents of different chemical nature, but of similar molecular weight. PS, as a porogen with a greater difference in the chemical composition between the polymeric porogen and PHEMA matrix than that of PMMA, formed larger pores, indicating a more pronounced phase separation despite almost identical solubility parameter values. This was accompanied by decreased specific surface area values and increased swellabilities in water. The effect of incompatibility appears here, leading to faster separation of the two polymer phases in the polymerization mixture when PS was used as a porogen. On the other hand, the use of PMMA probably led to a stronger interaction with the PHEMA molecules, producing a more homogeneous structure with a higher specific surface area.

Effect of crosslinking degree

The final porous structure of PHEMA depended not only on the degree of dilution (not treated here) and the extent of thermodynamic interactions between the diluent and network segments but also on the degree of crosslinking. It was an expanded gel at low EDMA contents (samples 1/I, 1/II). A macroporous copolymer was obtained only when the EDMA content and dilution were high. The effects of the EDMA percentage on swelling, pore volume and radius, and specific surface area are illustrated in Table I. Both the water

(swelling) and cyclohexane (pore volume) uptake increased with increasing EDMA content. Thus, the phase separation is enhanced for high EDMA contents, which is attributed to an increase in heterogeneity and stiffness of the network. A comparison of the pore volume values in Table I indicates that as the EDMA content increased from 2 to 40 wt %, the pore volume increased in samples prepared with a low molecular weight diluent. On the contrary, increasing the concentration of the crosslinking agent did not cause significant changes in pore volume values when the polymers were prepared with a high molecular weight porogen. An increase in the EDMA content promoted increased swelling, especially when the copolymer was formed in solvating diluents. An opposite fact was observed only in samples obtained with a 1,2-dichloroethane solution of PS porogen. The specific surface area increased and the average pore radius decreased with increasing degree of crosslinking. This is confirmed by comparing samples 3/II and 7/II in Figure 1(a), (b). Aggregates of globuli in sample 7/II (40 wt % EDMA) seem to be more packed than those in sample 3/II (2 wt % EDMA), which coincides with the smaller pore size of the former sample (Table I).

ES cell growth

As an example of applicability of PHEMA hydrogel to growth of undifferentiated mouse ES cells, a transparent sheet 1/II coated with gelatin was examined. After 48-h cultivation, no toxic effect of PHEMA hydrogel was observed. ES cells well survived and showed good morphology and adherence (Fig. 2). In both culture systems, the number of cells was roughly comparable. This was shown by fluorimetry through the use of the DNA-specific dye Hoechst 33258. Specifically, ES cells growing on a gelatinized culture plastic yield on average 388 arbitrary units and cells attached on gelatinized PHEMA fragments yield on average 350 arbitrary units. However, most colonies of ES cells growing on PHEMA hydrogel showed a typically less compact shape (Fig. 2). This may reflect the adaptation of ES cells to different diffusive and contact features of the PHEMA hydrogel. An ideal sheet for cell therapy should thus possess a pore size of at least 50 μm to be suitable for cell seeding. Design of such sheets is in progress.

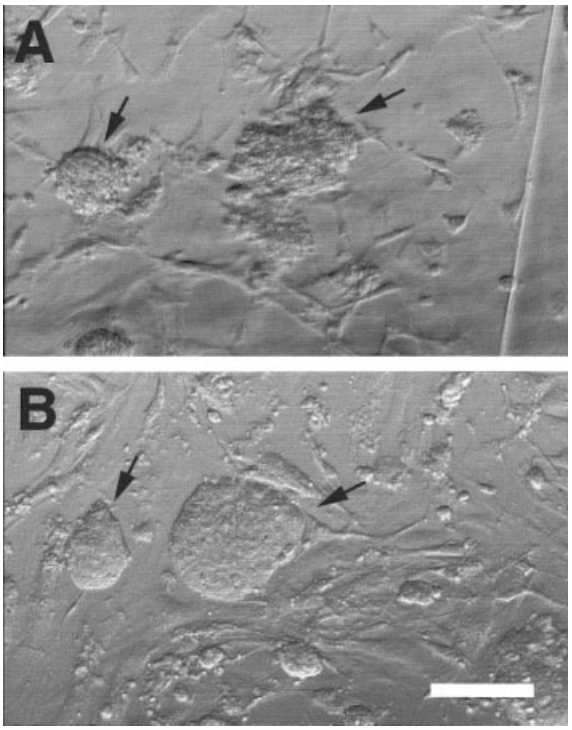

Figure 2 Mouse J1 ES cells at initial density of 8×10^4 per mL were plated either on a PHEMA sheet (A) or petri dish as a control (B) and grown for 48 h. Both surfaces were pretreated with 0.1 wt % gelatin. The arrows point to colonies of ES cells, which contain similar numbers of cells but are less compact in cell cultures on the PHEMA sheet. Bar represents 40 μm.

CONCLUSIONS

Depending on the type of the diluent (pore-forming agent) used and the concentration of crosslinking agent, the PHEMA sheets produced by bulk polymerization exhibited a wide range of pore volumes and average pore diameters. In the absence of diluent, homogeneous sheets resulted. The different behavior of diluents was explained by the differences in their solvating powers for the growing PHEMA chains during the network-formation process. A decrease in the solvating power of a diluent mixture increased the pore volume determined by cyclohexane regain at a low degree of crosslinking. An increase in the EDMA/HEMA ratio induced higher specific surface areas, pore volumes, and swellabilities. Addition of PMMA or PS to the polymerization mixture resulted in PHEMA sheets having large pores, the average radii of which varied in thousands of nm at a low degree of crosslinking. The highest pore volume up to 2 mL/g was obtained when the EDMA concentration in monomers and that of PMMA in the diluent phase was 40 and 20 wt %, respectively. The pore radius decreased with increasing concentration of a polymer porogen in 1,2-dichloroethane. In general, PS resulted in a larger pore size, increased swellabilities, and a lower surface area than those of PMMA. A pilot study indicated that PHEMA hydrogel sheets coated with gelatin supported the attachment and growth of ES cells and should be generally useful for the envisaged ES cell–based transplantation therapy.

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