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Creating porous tubes by centrifugal forces for soft tissue application

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Abstract

Chemically crosslinked poly(2-hydroxyethyl methacrylate) (PHEMA) tubes were synthesized by applying centrifugal forces to propagating polymer chains in solution. Initiated monomer solutions, with a composition typical for PHEMA sponges, were placed into a cylindrical mold that was rotated about its long axis. As polymerization proceeded, phase separated PHEMA formed a sediment at the periphery under centrifugal action. The solvent remained in the center of the mold while the PHEMA phase gelled, resulting in a tube. By controlling the rotational speed and the formulation chemistry (i.e., monomer, initiator and crosslinking agent concentrations), the tube dimensions and wall morphology were manipulated. Tube manufacture was limited by a critical casting concentration $[M]_c$, above which only rods formed. All tubes had an outer diameter of 2.4 mm, reflecting the internal diameter of the mold and a wall thickness of approximately 40–400 μm. Wall morphologies varied from interconnecting polymer and water phases to a closed cell, gel-like, structure. Concentric tubes were successfully prepared by using formulations that enhanced phase separation over gelation/network formation. This was achieved by using formulations with lower concentrations of monomer and crosslinking agent and higher concentrations of initiator. This technique offers a new approach to the synthesis of polymeric tubes for use in soft tissue applications, such as nerve guidance channels.

Keywords: Poly(2-hydroxyethyl methacrylate); High gravity; Hydrogel; Phase separation; Hollow fiber membrane; Guidance channel

1. Introduction

Tubes, and their fabrication, are of interest for applications including nerve guides, vascular grafts and cell encapsulation [1–3]. Of particular attraction are porous tubes, or hollow fiber membranes (HFMs), that serve as nerve guidance channels, providing a bridge between severed nerves [4–6]. Nerve guides have been manufactured using phase inversion [7], casting [8], extrusion [9,10], and dip-coating [11]. A new process of tube synthesis is presented in this paper that permits the formation of crosslinked, fully hydrated hydrogel tubes with a range of dimensions and morphologies unattainable using current processing techniques.

Rotating a cylindrical mold that is filled with a solution that can be phase separated during polymerization results in the creation of small diameter tubes. In this study, phase separation was induced by the aqueous solution polymerization of 2-hydroxy ethyl methacrylate (HEMA) [12,13]. Water is an excellent solvent for HEMA yet a poor solvent for poly(2-hydroxyethyl methacrylate) (PHEMA). As the propagating chain grows, its solubility in water decreases, leading to phase separation. Furthermore, PHEMA is denser than HEMA and water. Thus when HEMA is polymerized in a rotating cylindrical mold, phase separated PHEMA is pushed to the periphery of the mold by centrifugal forces, resulting in a tube with unique wall morphology.

While centrifugal forces have been previously used to create tubes, these tubes are formed at the liquid–air interface [14]. In this paper, we take advantage of a solution that is liquid–liquid phase separated during rotation for the creation of our tubes. Liquid–liquid phase separation of HEMA monomer, enriched with the propagating polymer chain, forms the denser phase relative to water. The monomer/polymer phase is neither liquid nor solid, but a viscoelastic phase. It is this phase that eventually gels and forms the tubular structure.

We describe this novel process to prepare tubular structures of PHEMA and detail how rotational speed

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and formulation chemistry—monomer, crosslinking agent and initiator concentrations—impact the morphology and dimensions of the tube formed.

2. Materials and methods

2.1. Materials

All chemicals were purchased from Aldrich (Milwaukee, WI) unless otherwise specified. Water was distilled and deionized using Millipore Milli-RO 10 Plus and Milli-Q UF Plus (Bedford, MA) at 18 MΩ resistance. All reactions were conducted at room temperature (RT). 2-Hydroxyethyl methacrylate (HEMA, 99 + %) and ethylene dimethacrylate (EDMA, 98%) were passed through a 10 ml glass pipette with 3 ml of monomethyl ether hydroquinone (MEHQ)—removing packing before use. Aqueous solutions of ammonium persulfate (APS) and sodium metabisulfite (SMBS) were used together as redox initiators.

2.2. Polymerization

HEMA was polymerized in the presence of excess water, with EDMA as a crosslinking agent, using an APS/SMBS redox initiating system. As detailed in Table 1, four series of hydrogel formulations were investigated for their effect on the synthesis of PHEMA tubes: monomer concentration (M-series), crosslinking agent concentration (X-series), initiator concentration (I-series) and rotational speed (S-series). Our shorthand notation, e.g. M-20-2700, indicates monomer series (M)—concentration of this variable (20 wt% monomer)—rotational speed (2700 rpm). The relevant quantities of HEMA, EDMA and water were added into a glass vial, and placed in an ultrasonic bath for 5 min. Sonication was repeated after addition of the APS solution. The appropriate volume of SMBS solution was added to this mixture, agitated by swirling and then sonicated for an additional 30 s. The monomer mixture was then drawn into a Luer-lok syringe using a 0.8 μm filter. The filter and needle were removed from the syringe and, using a new needle, the monomer mixture was injected into the polymerization molds. The time from addition of SMBS solution to rotation of the molds was less than 200 s.

As was indicated in Table 1, higher initiator concentrations were required for those formulations with monomer concentrations less than 10% HEMA. At higher HEMA concentrations, the APS/SMBS concentrations were 0.5/0.2 (w/w).

Table 1
Monomer mixture formulations

Formulation name/series	HEMA/H_2O wt% ratio	EDMA (wt% of monomer)	APS (wt% of monomer)	SMBS (wt% of monomer)	Rotational speed (rpm)
M-01	1:99	0.1	5[a]	2[a]	2700, 5000
M-03	3:97	0.1	1.67[a]	0.67[a]	2700, 5000
M-05	5:95	0.1	1[a]	0.4[a]	2700, 5000
M-07	7:93	0.1	0.71[a]	0.28[a]	2700, 5000
M-10	10:90	0.1	0.5	0.2	2700, 5000
M-15	15:85	0.1	0.5	0.2	2700, 5000
M-20	20:80	0.1	0.5	0.2	2700, 5000
M-25	25:75	0.1	0.5	0.2	2700, 5000
M-30	30:70	0.1	0.5	0.2	2700, 5000, 30 000
X-0	20:80	0	0.5	0.2	2700, 5000
X-1	20:80	0.1	0.5	0.2	2700, 5000
X-2	20:80	0.2	0.5	0.2	2700, 5000
X-3	20:80	0.3	0.5	0.2	2700, 5000
X-4	20:80	0.4	0.5	0.2	2700, 5000
X-5	20:80	0.5	0.5	0.2	2700, 5000
I-1	25:75	0.1	0.5	0.1	2700, 5000
I-2	25:75	0.1	0.5	0.2	2700, 5000
I-3	25:75	0.1	0.5	0.3	2700, 5000
I-4	25:75	0.1	0.5	0.4	2700, 5000
I-5	25:75	0.1	0.5	0.5	2700, 5000
S	20:80	0.1	0.5	0.2	1200, 1800, 2700, 5000, 10 000

[a]Formulations had higher APS/SMBS concentration to assist in polymerization.

2.3. Synthesis of PHEMA tubes

PHEMA tubes synthesized in custom-built disposable molds, are shown in Fig. 1a. Glass tubing (Kimble, Vineland, NJ) with an outer diameter (OD) of 4 mm and an inner diameter (ID) of 2.4 mm was cut to a 30 mm length with a tungsten-carbide knife. Tygon tubing (2.4 mm OD, 0.8 mm ID) was inserted 3 mm into each end of the glass tube and the outer portion removed with a scalpel. The monomer mixture was injected via a 20-gage needle from the lower end of the vertically held mold, thereby displacing all of the air within the mold. The top of the mold was then plugged with a stainless steel pin, the mold turned horizontal, the syringe withdrawn, and then capped, sealing the liquid-filled mold. The mold was placed in the chuck of an RZR-1 dual range, variable speed stirring drill (Heidolph, Germany) that had been mounted horizontally, using a spirit level. Polymerization proceeded in the rotating molds for a minimum of 5 h. For speeds of 5000 rpm and greater, a Dremel high-speed rotary tool (Model 398, Dremel, Racine, WI) was used. To accommodate the smaller chuck size of this drill, a different molding system was used, as shown in Fig. 1b. A small hole at one end of the bit was created to allow air to escape as the O-rings formed a seal. Drill speeds were accurately determined using a digital photo tachometer (Model 461893, Extech Instruments, Waltham, MA).

2.4. Tube dimensions

Tube dimensions were recorded with a Leica MZ-6 stereomicroscope, calibrated using an eyepiece graticule and a 0.1 mm grid. The tube dimensions were measured at the midpoints of three different tubes with two measurements of OD and ID taken at 90° per cross-section. Six dimensional measurements were therefore recorded, with numbers expressed as mean ± standard deviation. Tubes were removed from the glass molds and placed in water for at least one day before measurement. Tubes with less than 10 wt% HEMA were not removed from the mold, as they tended to collapse with handling.

2.5. Wall morphology

The morphology of PHEMA in the wall was examined with an environmental scanning electron microscope (ESEM), which permits the electron imaging of hydrated, uncoated samples in a more representative state [15,16]. Hydrated PHEMA tubes were sliced with a scalpel into tubes 0.2 mm in length and placed flat on the sample stage of the ESEM (Model E-2020, Electroscan Corporation, USA). An accelerating voltage of 20 kV was used in conjunction with a large spot size and magnifications less than ×1500, to limit both heating effects and sample damage. A working distance of 5–7 mm was employed to minimize the beam scattering. In the specimen chamber

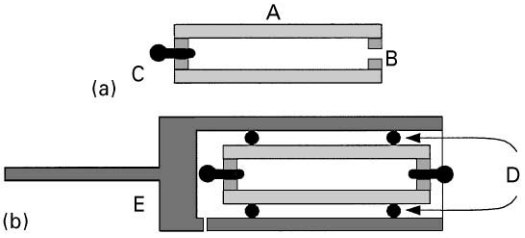

Fig. 1. Custom built mold to synthesize tubes consists of (a) glass pyrex tubing (A), Tygon tubing (B), stainless steel plugs (C); and (b) buna-N o-rings (D) and an aluminium bit (E), in which (a) is held for adaptation to different drill chuck sizes.

the pressure was maintained between 500 and 600 Pa and the temperature maintained at 1 ± 0.5°C using a Peltier-cooled sample stage. The sample chamber was periodically flushed with water vapor to maintain a satisfactory partial pressure of water, ensuring hydration of the hydrogel. Representative images of the wall morphology are reported.

2.6. Phase separation

Since the phase separation of PHEMA is an important part of PHEMA tube synthesis, turbidity was monitored for samples of *cast* PHEMA sponges using an Ultraspec 4000 spectrophotometer (Pharmacia Biotech, Canada). Timing began with the addition of SMBS, after which the formulation was sonicated for 30 s and then monitored for absorbance at 550 nm. Triplicate time-scans at 550 nm were performed for each formulation.

3. Results

The effects of four series of variables were investigated in terms of tube dimensions and morphology of the wall structure: monomer, crosslinking agent, initiator and rotational speed. The results of each are described below and correlated with phase separation/turbidity data.

3.1. Monomer (M) series

Higher monomer concentrations resulted in thicker walled tubes, as shown in Fig. 2, for both rotational speeds of 2700 and 5000 rpm. Tubes with 40 μm thick walls were prepared with monomer concentrations of 1 wt% (at 2700 rpm) and appeared as thin translucent films on the inner surface of the glass mold; these tubes were weak and tacky. Wall thickness was greatest for M-20-2700 with 385 ± 39 μm. Thus a broad range of wall thickness was possible, simply by varying monomer concentration.

Rotational speeds of 2700 and 5000 rpm did not significantly impact tube dimensions at monomer

concentrations between 3 and 15 wt%. The faster rotational speed resulted in thinner walled tubes only above 15 wt% HEMA, as shown in Fig. 2. A critical monomer concentration, $[M]_c$, was observed for the M-series, above which only solid rods were formed. At 2700 rpm, $[M]_c$ was between 20% and 25% whereas at 5000 rpm (and indeed at 30,000 rpm) $[M]_c$ was between 25% and 30%. The outside diameter of equilibrated tubes was similar to the ID of the glass mold (i.e., 2.4 mm) in which they were prepared, indicating little, if any, swelling/deswelling.

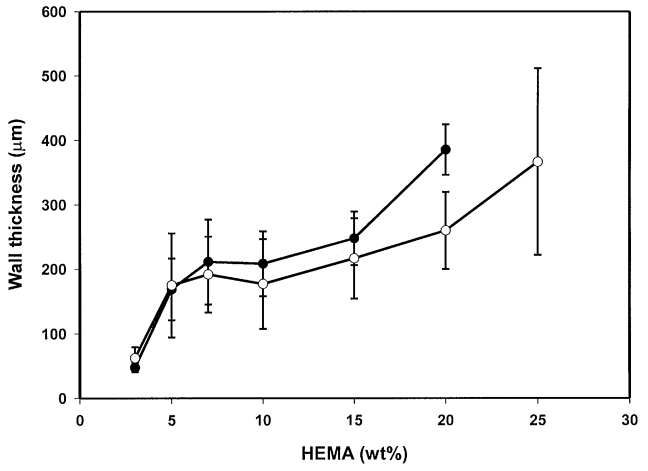

Fig. 2. Effect of monomer concentration on wall thickness for two rotational speeds: (●) 2700 rpm; (○) 5000 rpm.

The monomer concentration affected the microstructural geometry in the walls, as shown in Fig. 3. Both M-10-2700 (Fig. 3a) and M-15-2700 (Fig. 3b) were tubes, having predominantly gel-like wall structures of a continuous polymer phase (and discontinuous water phase). The individual droplets that phase-separated and spun to the periphery can be seen for M-20-2700 (Fig. 3c). At monomer concentrations higher than $[M]_c$ (i.e. M-25-2700, Fig. 3d), rods formed, having a different morphology than that observed for other tubes in this series. While one may postulate that higher rotations would result in tubes at these higher monomer concentrations, this was found to be untrue. For M-30, rods were formed even at 30,000 rpm.

To gain greater insight into the mechanisms of phase separation/gelation, turbidity measurements were taken. A more rapid increase in light absorbance (i.e. turbidity) was expected for polymerizations that promoted phase

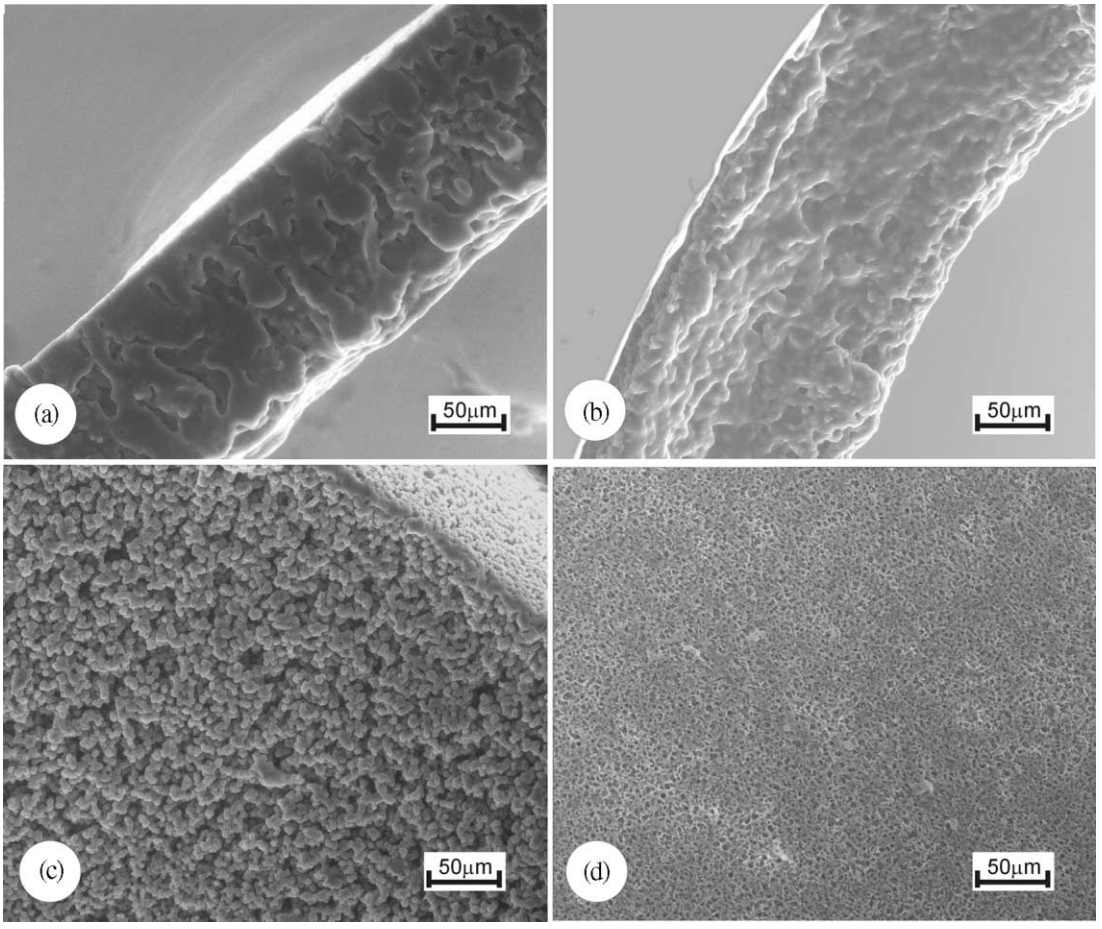

Fig. 3. ESEM micrographs of wall cross-sections for M-series: (a) M-10-2700, (b) M-15-2700, (c) M-20-2700 and (d) M-25-2700.

Table 2
Onset and turbidity times for cast PHEMA sponges

Formulation name	Turbidity onset ($time_{0.01}$) (s)	Turbidity time ($time_1 - time_{0.01}$) (s)	Resulting structure at 2700 rpm
M-15	792 ± 22	118 ± 25	Tube
M-20	647 ± 81	83 ± 6	Tube
M-25	889 ± 93	1227 ± 35	Rod
M-30	1302 ± 74	1629 ± 60	Rod
X-0	710 ± 67	90 ± 16	Tube
X-1	647 ± 81	83 ± 6	Tube
X-2	492 ± 46	80 ± 13	Tube
X-3	468 ± 92	67 ± 38	Tube
X-4	360 ± 63	35 ± 7	Rod
X-5	243 ± 42	20 ± 10	Rod
I-1	1727 ± 67	1715 ± 24	Rod
I-2	889 ± 93	1227 ± 35	Rod
I-3	771 ± 77	1095 ± 56	Tube
I-4	467 ± 60	575 ± 54	Tube
I-5	521 ± 60	297 ± 46	Tube

separation over gelation. As shown in Table 2 for the M-series, the times to reach onset (A = 0.01) and turbidity (time between A = 0.01 and A = 1) were shorter at lower monomer concentrations, when tubes formed (M-15-2700 and M-20-2700) than when rods formed (M-25-2700 and M-30-2700). Since the monomer is a solvent for the propagating polymer, greater monomer concentrations delayed phase separation, leading to greater wall thickness and sometimes rods instead of tubes. A possible explanation is that a transparent gel formed and was followed by microsyneresis, or the formation of a water phase within the polymer network.

3.2. Crosslinking agent (X) series

As shown in Fig. 4, higher concentrations of crosslinking agent led to thicker walled tubes and these were generally less concentric than those formed at lower crosslinking agent concentrations. Tubes were formed at all concentrations and at both rotational speeds except at 2700 rpm for higher EDMA concentrations (0.4 and 0.5 wt%). Tubes were formed at 5000 rpm (X-4-5000; X-5-5000) yet were not concentric, as reflected by large standard deviations.

All X-series samples exhibited porous wall structures, consisting of bicontinuous polymer droplets and water phases, as shown in Fig. 5. Interestingly, tubes prepared with low crosslinking agent concentrations exhibited some radial porosity within the wall (Fig. 5a). The morphology of rods in this series (Fig. 5b) was similar to that of tubes.

As summarized in Table 2 for the X-series turbidity measurements, increasing EDMA concentrations resulted in faster phase separation. As crosslinking agent

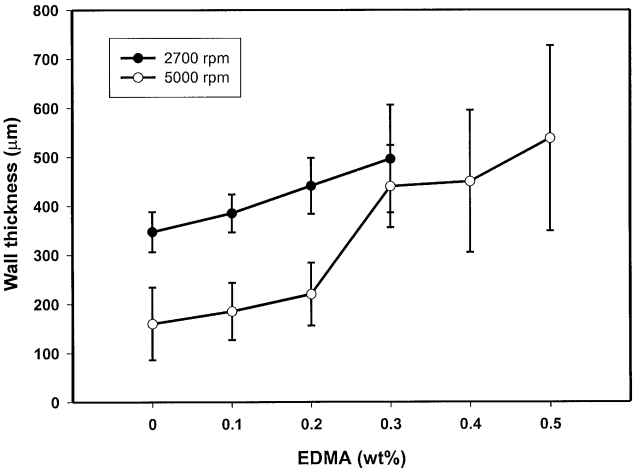

Fig. 4. Effect of crosslinking concentration on wall thickness for two rotational speeds: (●) 2700 rpm; (○) 5000 rpm.

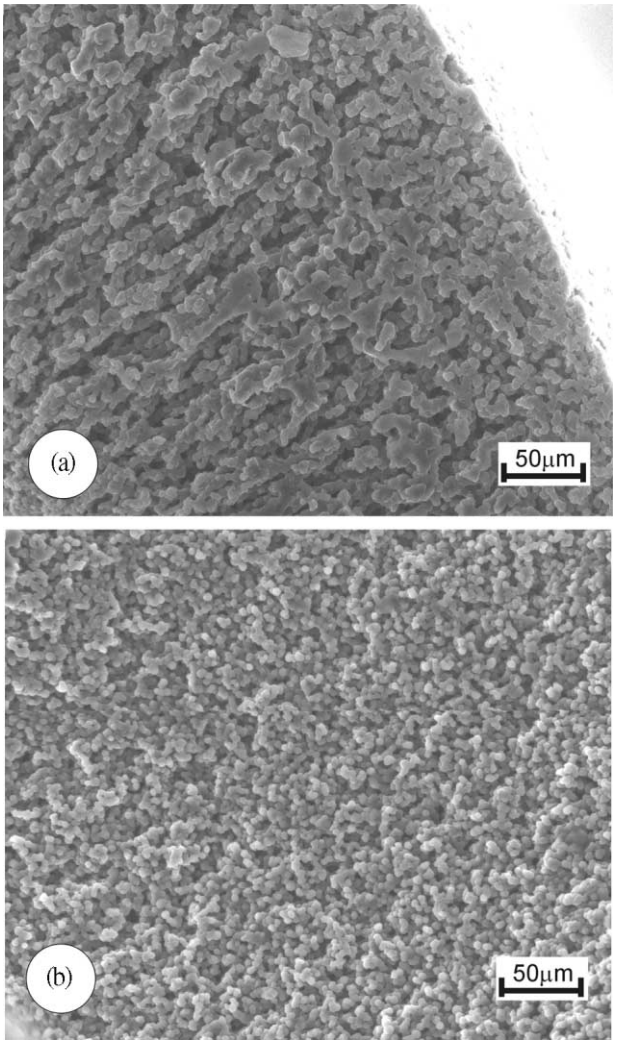

Fig. 5. ESEM micrographs of wall cross-section for X-series: (a) X-0-2700 and (b) X-5-2700.

concentration increased, both onset and turbidity times decreased. Greater concentrations of crosslinking agent result in accelerated polymer growth, accelerated monomer depletion and thus faster phase separation from 0 to 0.3% EDMA. The polymerization of HEMA is also exothermic, so increased polymerization rates generate more heat, which may be autocatalytic. At EDMA concentrations of 0.4% and 0.5%, rods formed instead of tubes likely because the centrifugal forces were insufficient to overcome the rapid gelation of PHEMA droplets.

3.3. Initiator (I) series

For the I-series, a minimum SMBS concentration of 0.3 wt% was required for tube formation at 2700 rpm. As shown in Fig. 6, as the SMBS concentration increased, more uniform, concentric PHEMA tubes were formed, having smaller standard deviations. Tubes were formed for all initiator concentrations at 5000 rpm, with concentricity improving at higher SMBS concentrations.

Changing the SMBS initiator concentration affected tube morphology. At 0.1% SMBS concentration, a bilayer structure formed, consisting of a porous rod surrounded by a gelled skin, as shown in Fig. 7a. The skin likely consisted of the initial phase separated PHEMA particles being pressed into a gel at the periphery. The remaining monomer formed a continuous network that can be seen in the core of the cross-section. At higher initiator concentrations (Fig. 7b), typical bicontinuous wall morphology was observed.

As shown in Table 2, both onset and turbidity times decreased with increasing SMBS concentrations for the I-series. With higher concentrations, the number of propagating radicals increased thereby consuming more monomer in a given time. Since the monomer helps to solubilize the polymer, the turbidity times decreased.

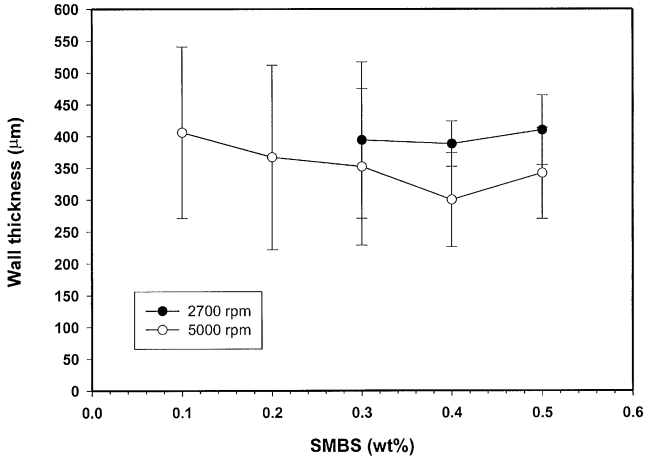

Fig. 6. Effect of SMBS concentration on wall thickness for two rotational speeds: (●) 2700 rpm; (○) 5000 rpm.

Given the correlation of polymer length, network formation and rods, it was not surprising that rods formed at lower SMBS initiator concentrations where fewer, longer chains propagated. At low SMBS concentrations rods formed probably because gelation resulted during the slow phase separation process, as in Fig. 7a. Onset and turbidity times were longer for rods than tubes, reflecting the longer times associated with gelation followed by microsyneresis.

3.4. Rotational speed (S) series

As rotational speed increased, the rod (at 1200 rpm) became a tube (at 1800 rpm) and wall thickness decreased to 260 μm (at 5000 rpm), after which there was no further decrease, as shown in Fig. 8. The most concentric tubes (i.e. lowest standard deviation with respect to wall thickness) formed at 2700 rpm. Sedimentation of PHEMA particles increased with rotational speed.

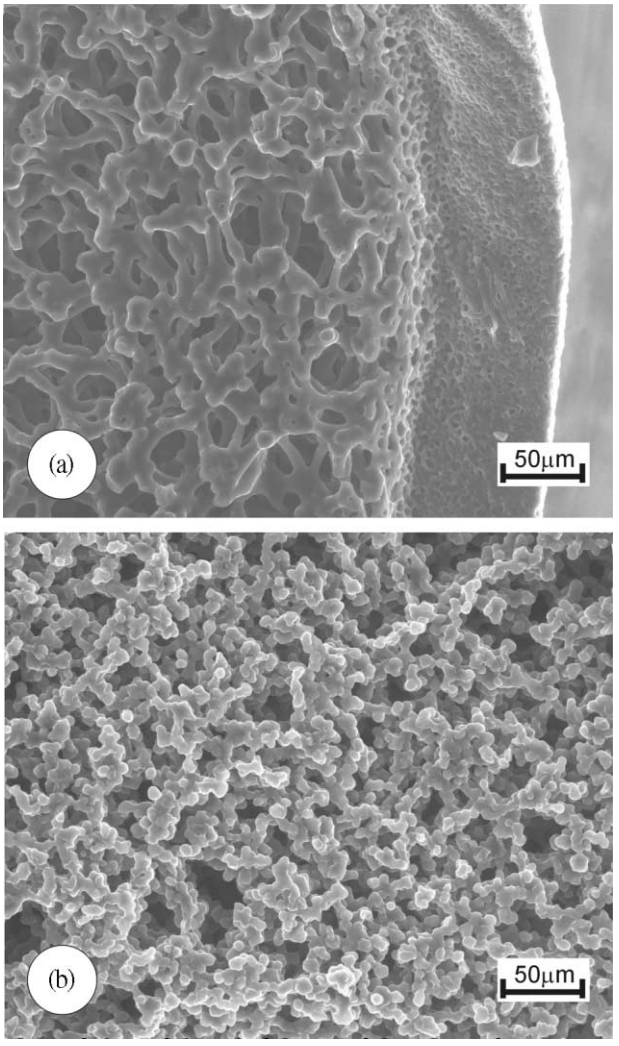

Fig. 7. ESEM micrographs of wall cross-section for I-series: (a) I-1-2700 and (b) I-5-2700.

Rotational speeds had a dramatic effect on the morphology of PHEMA tubes, as shown in Fig. 9. At lower speeds, S-1800 and S-2700, a particulate morphology was evident, having bicontinuous polymer and water phases (cf. Figs. 9a and b, respectively). At a higher rotational speed of 5000 rpm, the bicontinuous nature of the wall morphology appeared to diminish, having more of a gel-like morphology (S-5000, Fig. 9c). This cross-section is similar to that observed for lower monomer concentrations at lower rotational speeds (cf. Figs. 3a and b). The fastest speed of 10,000 rpm (Fig. 9d) resulted in a gel-like morphology, with three visible regions. The outer edge of the tube appeared porous but with a closed-cell morphology and limited connectivity. Approaching the inner part of the wall, a non-porous gel-like layer was formed, followed by a porous layer that lined the lumenal cavity of the tube.

4. Discussion

PHEMA has been previously shown to phase separate during propagation, leading to translucent or opaque hydrogels. A formulation phase diagram [17] for HEMA/EDMA/water is shown in Fig. 10. Homogenous gels form in Region A. Microporous phase-separated PHEMA (Region B) has closed cell water pores

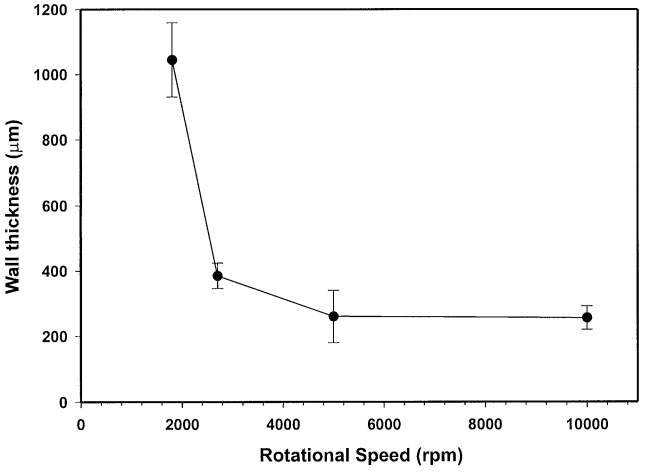

Fig. 8. Effect of rotational speed on wall thickness for 20%HEMA (S-series).

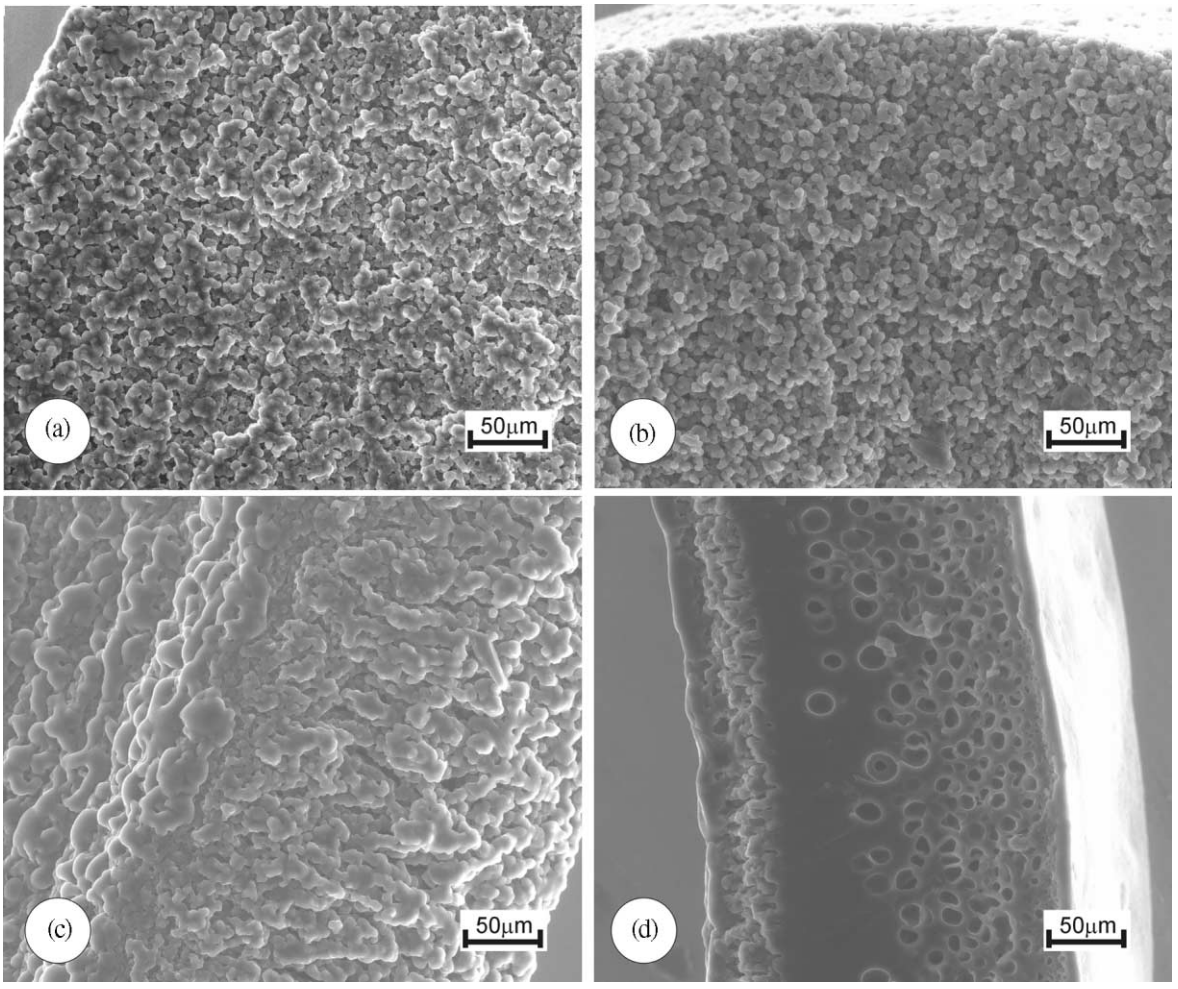

Fig. 9. ESEM micrographs of wall cross-section for S-series: (a) S-1800, (b) S-2700, (c) S-5000 and (d) S-10000.

surrounded by a continuous polymer phase morphology. Bicontinuous scaffolds that permit cell invasion are termed macroporous phase-separated PHEMA sponges (Region C). The immiscible HEMA/EDMA/water region is that of D. Tubes are formed herein when centrifugal forces are applied to a formulation as described in Region C.

Taking advantage of the manner in which macroporous phase separated PHEMA sponges form during polymerization, we created tubes by rotation and found that rotational speed and the concentrations of monomer, initiator and crosslinking agent all influence the resulting geometry and morphology. As might be expected, those variables that increased the rate of polymer and network formation—i.e., concentrations of monomer and crosslinking agent—resulted in either thicker walled tubes or rods. As rotational speed increased, thinner walled tubes resulted for most formulations, reflecting the greater centrifugal force applied to the denser monomer/polymer phase.

Tubes (as opposed to rods) resulted when phase separation occurred before gelation (Region C in Fig. 10); however, it was possible to create tubes having gel-phase wall structures similar to that observed in Region B through compaction. Below the $[M]_c$ (<25 wt%), tubes were formed whereas above $[M]_c$, rods were formed. These concentrations reflect the change from macroporous to microporous regions (Regions C to B) of phase-separated PHEMA. The monomer solubilized the propagating polymer chains, with higher concentrations of monomer resulting in increased solubility of the polymer. The wall morphology that resulted had a series of PHEMA spheres or droplets that formed either a continuous polymer gel-like phase or bicontinuous polymer and water phases. Thus with polymerization during rotation there are three competing factors that influence the resulting geometry: phase separation, gelation and centrifugal force. If phase separation precedes gelation, tubes result; if gelation precedes phase separation, rods result.

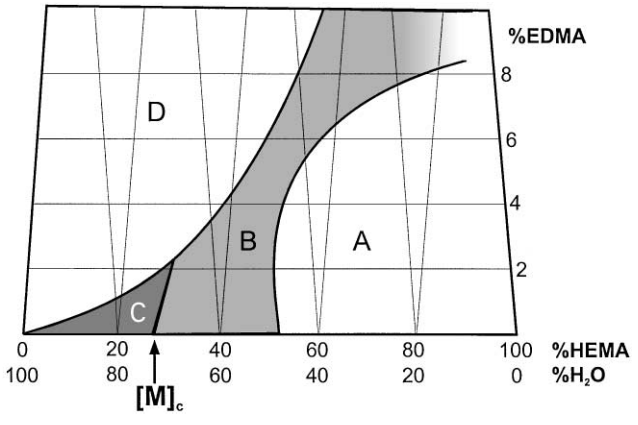

Fig. 10. Regions of formation for homogeneous (A), microporous (B), and macroporous (C) PHEMA. The immiscible region of the three components is D. Image adapted from Šprincl et al. [17] and modified showing critical monomer concentration $[M]_c$.

5. Conclusion

PHEMA tubes were synthesized by rotating a mold filled with a liquid that phase separates. By controlling the rotation speed and rate of liquid–liquid phase separation (monomer, initiator, crosslinking agent concentrations), the resulting geometry and wall morphology were manipulated. PHEMA tubes were prepared by using formulations that enhanced liquid–liquid separation; achieved with lower concentrations of monomer and crosslinking agent and higher concentrations of initiator. When gelation preceded phase separation, specifically those above $[M]_c$, these formulations resulted in rods.

The technique outlined herein offers a new approach to synthesize crosslinked hydrogel tubes that can be either porous or non-porous in nature. Furthermore, very little material was required to create the tubes. This is especially useful in biomedical applications where high value material is often in short supply. We are currently evaluating the utility of these tubes for guided regeneration in the nervous system.

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References

[1] Chandy T, Das GS, Wilson RF, Rao GHR. Use of plasma glow for surface-engineering biomolecules to enhance blood compatibility of Dacron and PTFE vascular prosthesis. Biomaterials 2000;21:699–712.

[2] Rodriguez FJ, Gomez N, Perego G, Navarro X. Highly permeable polylactide-caprolactone nerve guides enhance peripheral nerve regeneration through long gaps. Biomaterials 1999;20: 1489–500.

[3] Gentile FT, Doherty EJ, Rein DH, Shoichet MS, Winn SR. Polymer science for macroencapsulation of cells for central nervous system transplantation. Reactive Polymers 1995;25:207–27.

[4] Cao X, Shoichet MS. Delivering neuroactive molecules from biodegradable microspheres for application in central nervous system disorders. Biomaterials 1999;20:329–39.

[5] Ramon-Cueto A, Plant GW, Avila J, Bunge MB. Long-distance axonal regeneration in the transected adult rat spinal cord is promoted by olfactory ensheathing glia transplants. J Neurosci 1998;18:3803–15.

[6] Guest JD, Rao A, Olson L, Bunge MB, Bunge RP. The ability of human Schwann cell grafts to promote regeneration in the transected nude rat spinal cord. Exp Neurol 1997;148:502–22.

[7] Shoichet MS, Rein DH. In vivo biostability of a polymeric hollow fibre membrane for cell encapsulation. Biomaterials 1996;17:285–90.
[8] Chamberlain LJ, Yannas IV, Hsu HP, Strichartz G, Spector M. Collagen-GAG substrate enhances the quality of nerve regeneration through collagen tubes up to level of autograft. Exp Neurol 1998;154:315–29.
[9] Widmer MS, Gupta PK, Lu LC, Meszlenyi RK, Evans GRD, Brandt K, Savel T, Gurlek A, Patrick CW, Mikos AG. Manufacture of porous biodegradable polymer conduits by an extrusion process for guided tissue regeneration. Biomaterials 1998;19:1945–55.
[10] Borkenhagen M, Stoll RC, Neuenschwander P, Suter UW, Aebischer P. In vivo performance of a new biodegradable polyester urethane system used as a nerve guidance channel. Biomaterials 1998;19:2155–65.
[11] Meek MF, Dijkstra JR, Den Dunnen WFA, Ijkema-Paassen J, Schakenraad JM, Gramsbergen A, Robinson PH. Functional assessment of sciatic nerve reconstruction: Biodegradable poly(DLLA-epsilon-CL) nerve guides versus autologous nerve grafts. Microsurg 1999;19:381–8.
[12] Lou X, Dalton PD, Chirila TV. Hydrophilic sponges based on 2-hydroxyethyl methacrylate—Part VII: modulation of sponge characteristics by changes in reactivity and hydrophilicity of crosslinking agents. J Mater Sci—Mater Med 2000;11:319–25.
[13] Dalton PD, Vijayasekeran S, Shoichet MS. Polymerization. In: Atala A, Lanza R, editors. Methods of tissue engineering. San Diego: Academic Press, 2001, in press.
[14] Kuzma P, Moro DG, Quandt H. Preparation of homogeneous hydrogel copolymers. US Patent #5,266,325, 1993.
[15] Danilatos GD. Introduction to the ESEM instrument. Microsc Res Tech 1993;25:354–61.
[16] Clayton AB, Chirila TV, Dalton PD. Hydrophilic sponges based on 2-hydroxyethyl methacrylate. 3. Effect of incorporating a hydrophilic crosslinking agent on the equilibrium water content and pore structure. Polym Int 1997;42:45–56.
[17] Šprincl L, Kopecek J, Lim D. Effect of the structure of poly(glycol monomethacrylate) gel on the calcification of implants. Calcif Tissue Res 1973;13:63–72.