Poly(ethylene oxide)-Grafted Thermoplastic Membranes for Use as Cellular Hybrid Bio-Artificial Organs in the Central Nervous System

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Poly(acrylonitrile-co-vinyl chloride) (PAN/VC) anisotropic membranes were chemically modified with poly(ethylene oxide) (PEO) (5000 and 20,000 g/mol) by one of two aqueous reactions: (a) acid hydrolysis of the nitrile group to a carboxylic acid with which amine-terminated PEO (PEO–NH_2) reacted or (b) base reduction of the nitrile group to an amine with which PEO–succinimide (PEO–SC) reacted. Approximately 1.3% of the bulk material was modified with PEO–NH_2 whereas 1.8 to 3.5% was modified with PEO–SC as determined by proton nuclear magnetic resonance (^{1}H NMR) and attenuated total reflectance Fourier transform infrared (ATR FTIR) spectra. Approximately 50 to 75% less bovine serum albumin (BSA) adsorbed to PEO-grafted single skin fibers than to unmodified PAN/VC. Transport properties of modified and unmodified fibers were compared by passive diffusion, convective nominal molecular weight cutoff, and hydraulic permeability. Neither hydraulic permeability nor nominal molecular weight cutoff of BSA changed appreciably after surface modification with PEO indicating that pore structure was not adversely affected by the chemistry involved in grafting poly(ethylene oxide). However, in the absence of any membrane conditioning, the apparent diffusion of α-chymotrypsinogen (24,000 g/mol) was enhanced in PEO-grafted PAN/VC fibers possibly as a result of reduced sorption of the permeating protein. In vivo biocompatibility in the brain tissue of rats was judged by histological assessment of the host's cellular response to fibers implanted for 30 days; biocompatibility of both PAN/VC and PAN/VC–g-PEO was satisfactory but improved slightly with PEO grafting.

Key words: surface modification • biocompatibility • artificial organs • biohybrid

INTRODUCTION

Poly(acrylonitrile-co-vinyl chloride) (PAN/VC) hollow fiber membranes have been used to encapsulate living cells that release bioactive products for the treatment of serious diseases and disabilities such as type I diabetes, Parkinson's disease, and chronic pain.[1,7,17,22] Such membranes restrict passage of cytotoxic agents and are compatible with surrounding host tissue. Although investigators have reported good biocompatibility of PAN/VC implants,[7,24] the design and handling of PAN/VC implants are limited in versatility. For example, the composition of cell culture medium to which fibers (and devices) may be exposed prior to implant is restricted. Unlike a PAN/VC fiber immersed in a chemically defined medium prior to implant, a fiber immersed in a medium containing serum of a xenogeneic source evokes a host–tissue reaction in the brain and other sites. In modifying the surface of PAN/VC, the design and handling of such implants may be facilitated. By decreasing protein adsorption, for example, the devices may be immersed in a serum-containing medium which may extend their shelf life. Accordingly, attempts are made to enhance the biocompatibility of PAN/VC membranes by chemically grafting poly(ethylene oxide) (PEO) to preformed PAN/VC hollow fiber membranes of two distinct morphologies. Chemically modifying the preformed membrane is more desirable than changing the membrane's formulation because the latter complicates the process of membrane fabrication and optimization of the membrane's properties such as size, configuration, and transport.

Several approaches have been taken to improve the biocompatibility of implant-grade material; for example, surface modification techniques used to inhibit protein adsorption include the introduction of negative charge and coating the surface with a biologically derived compound, among others.[12] Poly(ethylene oxide) offers unique properties for biomedical and biotechnical applications.[14] In PEO-grafted materials the bulk mechanical properties of the solid material remains unchanged whereas the surface acquires the properties of PEO, thereby producing a surface that may be better suited for in vivo applications. PEO-modified surfaces resist protein adsorption likely because PEO chains exclude a volume as a result of their mobility and because PEO is hydrophilic and neutral.[9] For example, grafting PEO to poly(vinyl chloride) was reported to improve antithrombogenic properties without affecting the mechanical properties,[20] a similar effect was observed with PEO-copolymerized materials.[3] Poly(ethylene oxide)-grafted polystyrene exhibits decreased adsorption of fibrinogen relative to the unmodified material.[4] The surface

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of PEO-grafted polyethylene (PE–PEO) was reported to be more hydrophilic than unmodified PE and resembles that of a hydrogel; PE–PEO decreased, relative to PE, the adsorption of human transferrin by 11 times.[2] To our knowledge, surface modification of PAN/VC membranes has not been reported.

Poly(ethylene oxide) surface modifications[5,13] can be achieved by a number of techniques; herein two methods to graft PEO to poly(acrylonitrile-co-vinly chloride) (PAN/VC) membranes are described (Schemes 1a and b). The nitrile group of PAN/VC is targeted for derivatization by either acid hydrolysis or base reduction. By acid hydrolysis, the nitrile is converted to a carboxylic acid group with which end-functionalized PEO–amine[4,26] (PEO–NH_2) reacts via a carbodiimide-mediated coupling reaction. By base reduction, the nitrile group is converted to a primary amine group with which end-functionalized PEO–succinimide[9,16,18,26] (PEO–SC) reacts. The derivatized PEO is dissolved in an aqueous solution in which PAN/VC is insoluble to limit the reaction to the surface region of PAN/VC and to avoid changing the properties of the preformed hollow fiber membrane.

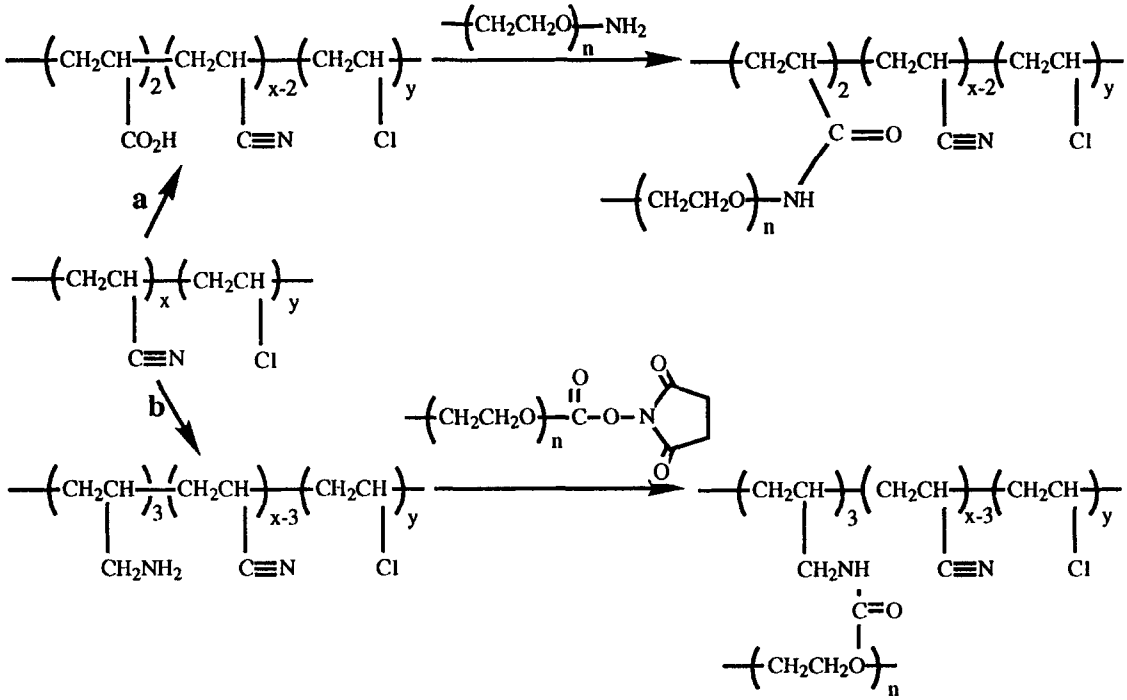
PAN/VC–g-PEO membranes were characterized in terms of both transport and biocompatibility. Transport was measured convectively by nominal molecular weight cutoff (nMWCO) and passively by diffusion. Biocompatibility was predicted by a protein adsorption assay with bovine serum albumin (BSA) and assessed in vivo by the composition of reactive cells around an implanted PAN/VC or PEO-modified PAN/VC hollow fiber membrane in the brain. In this article, PEO represents both higher and lower molecular weights of PEO, the latter of which is often referred to as poly(ethylene glycol).

MATERIALS AND METHODS

Monosuccinimide-terminated PEO (PEO–SC, 8000 and 20,000 g/mol) and monoamine-terminated PEO (PEO–NH_2, 5000 and 20,000 g/mol) were prepared at Shearwater Polymers (Huntsville, AL); all chemicals used for derivatization were purchased from Aldrich. BBL FTA hemagglutination buffer (FTA) was purchased from Becton Dickinson (Cockeysville, MD) and prepared by dissolving 9.23 g of FTA powder in 1 L of MilliQ water (Waters Corporation, Milford, MA). Dextrans T10, T40, T70, and T500 were purchased from Pharmacia (Uppsula, Sweden) and dissolved in FTA buffer to make a 0.5% solution. Bovine serum albumin (fraction V) and blue dextran were purchased from Sigma (St. Louis, MO). A 0.4-mg/mL solution of BSA and a 0.4 mg/mL solution of blue dextran were both prepared in FTA. Dimethyl sulfoxide (DMSO, molecular biology grade) was purchased from Fisher (Fairlawn, NJ).

Hollow Fiber Membranes

The PAN/VC ultrafiltration grade hollow fiber membranes were prepared at CytoTherapeutics using either a dry–wet (single-skin) or wet–wet (double-skin) spinning process with a hollow fiber spinneret.[6] Single-skin membranes are composed of a selectively permeable inner skin, a finger-like wall structure, and a relatively rough porous



Scheme 1. *Derivatization of PAN/VC by* (a) hydrochloric acid/sodium hydroxide hydrolysis and subsequent reaction with amine-terminated PEO (PEO–NH_2) and (b) sodium borohydride reduction and subsequent reaction with succinimide-terminated PEO (PEO–SC).

outer surface. Double-skin membranes are composed of selectively permeable inner and outer skins with a finger-like wall structure. Scanning electron micrographs of both single-skin and double-skin hollow fiber membranes are included in Figure 1. The dimensions of the single-skin fibers are 650 ± 10 μm inner diameter, 750 ± 10 μm outer diameter. Double-skin fibers are 1150 ± 30 μm inner diameter, 1740 ± 40 μm outer diameter. Hollow fibers were soaked in a 25% aqueous glycerol (Fisher) solution and then dried prior to derivatization. The chemically modified fibers were stored in FTA buffer containing 0.05% sodium azide (Aldrich).

Chemical Modification

Poly(ethylene oxide)-grafted PAN/VC hollow fiber membranes were characterized for the presence of PEO and compared to PAN/VC and PAN/VC-modified membranes by both attenuated total reflectance Fourier transform infrared (ATR FTIR) spectra and proton nuclear magnetic resonance (^{1}H NMR).

Hydrolysis of PAN/VC (PAN/VC–CO_2H)

PAN/VC fibers were added to 10 mL of concentrated hydrochloric acid (Aldrich, Milwaukee, WI) (48 h, room temperature [rt]). The fibers were washed with distilled water and then 10 mL of 10 *M* sodium hydroxide (48 h, rt). The resulting fiber product was washed with water until neutral and then 5% oxalic acid (10 m), water, and finally 95% ethanol.

Reaction of PAN/VC–CO_2H with PEO–NH_2 (PAN/VC–PEO–NH_2)

PAN/VC–CO_2H was added to a beaker containing 0.7 g of methoxy-terminated poly(ethylene oxide)-amine (MPEO–NH_2) (5000 or 20,000 g/mol), 0.28 g *N*-3-dimethylaminopropylethyl carbodiimide, and 7 mL of water. The pH of the solution was adjusted to pH 4.5 with dilute HCl and heated (40°C, 5 h); the fibers were washed with water, 95% ethanol (40°C, 48 h), and then water. As a control reaction, unmodified PAN/VC was exposed to PEO–NH_2 (PAN/VC + PEO–NH_2) under similar conditions.

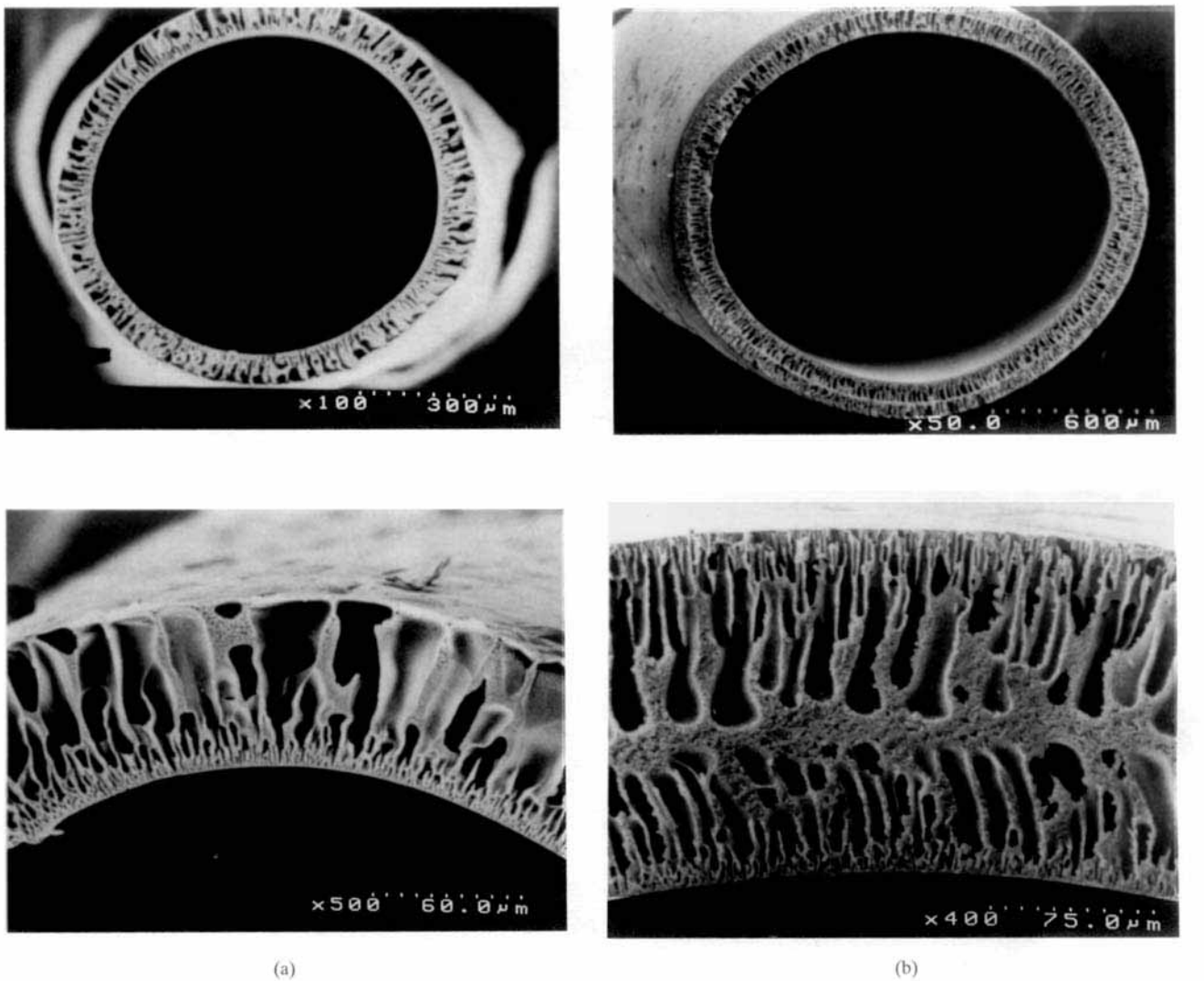


Figure 1. Scanning electron micrographs of PAN./VC hollow fiber membranes: (a) single skin (100×; 500×) and (b) double skin (400×; 50×).

Reduction of PAN/VC (PAN/VC–NH_2)

PAN/VC fibers were added to an aqueous solution of 10% sodium borohydride (0.7 g $NaBH_4$/7 mL H_2O) (48h, rt) and washed with water followed by 95% ethanol.

Reaction of PAN/VC–NH_2 with PEO–SC (PAN/VC–PEO–SC)

Amine-functionalized PAN/VC (PAN/VC–NH_2) was added to a beaker containing 7 mL of a 0.1 *M* sodium phosphate buffered solution (pH 8). To this solution was added 0.7 g of monosuccinimidyl carbonate–terminated poly(ethylene oxide) (PEO–SC) (8000 or 20,000 g/mol), (40°C, 48 h). The fiber product (PAN/VC–PEO–SC) was washed with water, 95% ethanol (40°C, 48 h), and then water. As a control reaction, unmodified PAN/VC was exposed to PEO–SC (PAN/VC + PEO–SC) under similar conditions.

Analytical Techniques

Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained on a 200-MHz Bruker instrument with 4 to 5 mg of polymer dissolved in deuterated acetone. All peaks were relative to that of acetone. Attenuated total reflectance Fourier transform infrared spectra (ATR FTIR) were obtained on a Digilab FTS-60A spectrometer using a germanium crystal (45°) to which the fibers were compressed. The ATR FTIR and ^{1}H NMR spectra were taken of PAN/VC flatsheet membranes which were prepared by casting a film on glass and into water. Ultraviolet spectra were attained on a Hitachi U-2000. Gel permeation chromatographs (GPC) were obtained using 8 μm gel filtration chromatography (GFC) columns (Polymer Laboratories, United Kingdom) with an L6000 Hitachi pump and a L-3350 Hitachi refractive index detector. Scanning electron micrographs (SEM) were taken on a Hitachi 2700. Histological sections of the brain and capsule were stained with hematoxylin (Gills, Fisher) and eosin (Y, Sigma) (H&E) and antibodies to glial fibrillary acidic protein (GFAP, Dako). Samples were cryosectioned with a Cryo-Cut microtome (American Optical Corporation).

Nominal Molecular Weight Cutoff

A known number of glycerinized, air-dried fibers were potted in a testing device cartridge using 5-min epoxy. Glycerin was removed from the fibers by ultrafiltration with MilliQ water (Waters, Milford, MA) at 5 psig transmembrane pressure (TMP) after which the fibers were rinsed with 1000 mL FTA buffer. The hydraulic permeability (expressed in mL/min/m^2/mm Hg) was then determined by time collection of filtrate samples at 5 psig. The nMWCO test began by recirculating a 300-mL solution of either BSA (0.4 mg/mL) or polydisperse dextran (0.5% solution of T10, T40, T70, T500). Ultrafiltrate and reservoir samples were taken at t values of 0 (before recirculating), 15, 30, and 45 min for BSA and after 80 min for polydisperse dextran. After testing, the fibers were challenged with a 0.4-mg/mL solution of 2 million molecular weight blue dextran as a check for leaks. Rejection coefficients (%R) were then calculated from the equation

$$\%R = [1 - (\mathrm{UF}/\mathrm{RES})] \times 100 \qquad (1)$$

where UF is the concentration of the ultrafiltrate and RES is that of the reservoir at a given time point. The BSA filtrate and reservoir concentrations were measured by absorbance at 281 nm. With the polydisperse dextran solution, filtrate and reservoir concentrations were determined as a function of molecular weight from gel permeation chromatography (GPC).[19]

Diffusion Test

Glycerin was removed from fibers by immersion in FTA buffer solution (2 days, rt). After draining excess buffer, 40 μL of a 3-mg/mL solution of α-chymotrypsinogen in FTA was loaded by pipette into the lumen of each fiber. The fibers were then sealed, rinsed briefly in FTA, and then inserted vertically into 6 mL of FTA in a 15-mL conical centrifuge tube. Samples of the FTA/diffusate bath were collected over a 24-h period; the fluid in the tubes was maintained at 37°C and the volume was maintained at 6 mL by replenishing after sampling. The bath was replenished with fresh buffer after each sample was removed. Device diffusion coefficients were then calculated using a Fick's law analysis with boundary layers corrected by a Sherwood number analysis. These tests were repeated on cohorts of fibers which had been stored in a solution containing 4 mg/mL insulin (37°C, overnight) prior to testing. The diffusate was analyzed for α-chymotrypsinogen by either high-performance liquid chromatography (HPLC) or UV absorbance. (This work was described by Dionne, et al. at the American Society of Artificial Internal Organs, April, 1993).

Protein Adsorption Assay

A 5% solution of BSA was prepared by dissolving 5 g of BSA in 100 mL of FTA solution with stirring. Three 1-cm-long fibers were immersed in 3 mL of 5% BSA solution in a six-well plate (37°C, 3 days). The fibers were removed and washed with FTA (5 times, 1 h) and then assayed by the bicinchonic acid (BCA) test (Pierce, Rockford, IL) for protein adsorption. In the BCA test, proteins reduce Cu(II) to copper (I), which then complexes with BCA forming a colored complex, the absorbance (at 562 nm) of which varies in proportion to the amount of proteins present. The BCA assay was performed directly on the PAN/VC and PAN/VC–PEO fibers (2 h, rt). The amount of protein adsorbed was determined by sampling the BCA solution in which the fibers had been immersed and analyzing this solution colorimetrically. The UV absorbance of the BCA protein solution was read on

an enzyme-linked immunosorbent assay (ELISA) (molecular Devices Thermo Max) microplate reader at 570 nm. The quantity of adsorbed protein was calculated by comparison of supernatant to a calibration curve for BSA (μg/mL); a nominal surface sorption (μg/cm^2) was then calculated using the external surface area of the fiber calculated using πdl where d is the outer diameter and l is the length.

Biocompatibility

The host reaction (i.e., biocompatibility) to PEO-grafted PAN/VC was assessed by implanting fibers (6 mm in length and 0.8 mm in width) into the brains of Lewis rats (~250 g). Prior to implantation, fibers were sterilized by immersion in 70% ethanol overnight and then stored in sterile Hanks' balanced salt solution (HBSS) for 48 h. The fibers were sealed and held at 37°C in serum-free HL-1 medium (Hycor, Portland, ME) for 2 days; fibers were then rinsed in sterile HBSS twice prior to implant. Four fibers of each type were stereotaxically implanted in 16 animals into the striatum (unilaterally) of a rat brain at the following coordinates: 0.5 mm anterior to bregma, 3.8 mm lateral to the sagittal suture, and 7.5 mm below the cortical surface.[10] After 4 weeks, the rat was sacrificed and the brain was perfused with 4% paraformaldehyde; it was then cryosectioned at 20 μm and assessed for reactive cells by H&E and immunostained for reactive astrocytes with antibodies to GFAP as previously described.[10] Approximately 30 sections were prepared for each sample, five levels of which were examined histologically.

Biocompatibility was scored according to the presence of macrophages (MACs), foreign body giant cells (FBGCs), polymorphonuclear leukocytes (PMNs), eosinophils (EOs), and GFAP-immunoreactive astrocytes: 1 indicates an acceptable response, 2 an intermediate response, and 3 an unacceptable response. Previous experiments have established criteria for an acceptable response based on Sham surgical controls. (See Winn et al.[25]) Following this response rating system, the overall biocompatibility of the fiber was determined in a single "blind" mode, an overall rating of 1 representing the best and 3 the least biocompatibility. The biocompatibility of cell-loaded fibers was assessed in a similar manner as described for empty capsules. Fibers were loaded with PC12A cells (rat pheochromocytoma, strain 12A) suspended in a chitosan slurry (SeaCure, Pronova, Norway), sealed, and then treated as empty capsules prior to implantation.

Statistical Evaluation

A Mann-Whitney U-statistical test[15] was performed on the data in Tables I and II: the p values that are tabulated indicate whether a statistically significant difference exists between raw data. For $p \leq 0.05$, the difference is significant; otherwise, it is not statistically significant. StatView (Abacus Concepts, 1991) was used to automate the analysis. The reported p values in Tables II and IV compensate for the occurrence of ties in the relative ranking of two populations.

RESULTS

Figure 2 contains the ATR FTIR spectra for the reaction of PEO–NH_2 with PAN/VC–CO_2H. In the spectra, it is evident that the nitrile group (2238 cm^{-1}) was hydrolyzed via an amide group (1656 cm^{-1}) to a carboxylic acid group (1762 cm^{-1}). Despite incomplete conversion of the nitrile group to carboxylic acids, sufficient carboxylic acid groups were formed to allow the attachment of PEO–amine (1110 cm^{-1} = ν_{C-O}). By ^{1}H NMR, PEO is evident in the PAN/VC spectrum as a result of the appearance of the peak at 3.61 ppm; the bulk yield of the reaction is 1.3%. Given that ATR IR spectra probe the outer micrometer of a polymer surface, it is likely that the PEO present is grafted to the outer surface.

Figure 3 follows the reaction of PEO–SC with PAN/VC–NH_2 by ^{1}H NMR. The peak ratio of vinyl chloride (4.43 ppm) to acrylonitrile (3.39 ppm) protons of PAN/VC increases by 134% after reduction to PAN/VC–NH_2, indicating conversion of the nitrile group to a primary amine; a successful grafting reaction with PEO–SC is evidenced by the peak at 3.61 ppm and a bulk yield of 1.8 to 3.5%. The reaction with 20,000 g/mol PEO–SC indicates that 5.5% of the bulk consists of PEO. By ATR FTIR spectra, the nitrile group (2238 cm^{-1}) of PAN/VC was reduced to an amine group (3700 cm^{-1} ν_{N-H}) forming PAN/VC–NH_2 which then reacted with PEO–succinimide (8000 g/mol) (1109 cm^{-1} ν_{C-O}).

The ^{1}H NMR and ATR FTIR spectra for the control grafting experiments indicate that most of the PEO–NH_2 and the PEO–SC is removed by the wash procedure. By ^{1}H NMR, the control experiment for PEO–SC (20,000 g/mol) shows that 1.1% of the bulk is attributed to PEO–SC whereas 5.5% of the bulk is attributed to PEO–SC for the grafted PAN/VC–PEO–SC (see Figures 2 and 3).

Data for BSA and dextran nMWCO and hydraulic permeability are presented in Table I. Figure 4 shows the

Table I. Nominal molecular weight cutoff (nMWCO) and hydraulic permeability data comparing PEO-grafted and unmodified PAN/VC membranes.

Sample	nMWCO (~95% rejection)		Hydraulic permeability (mL/m^2/min/mm Hg)
	Protein (BSA)	Dextran	
PAN/VC	36.1 Å/67,000 g/mol	65 ± 10 Å	7.0 ± 20%
PAN/VC–PEO–NH_2	36.1 Å/67,000 g/mol	75 ± 10 Å	5.8 ± 20 Å

Table II. Comparison of amount of protein adsorbed to PAN/VC–g-PEO and PAN/VC.

Sample (n = 3)	Protein adsorbed (BSA) (mg/cm^2)[a]	p Values comparing unmodified PAN/VC to modified fibers[b]
Single-skin membranes		
Unmodified: PAN/VC	57 ± 5	
Derivatized with PEO		0.05
PAN/VC–PEO–NH_2 (5k)	29 ± 12	
PAN/VC–PEO–SC (20k)	14 ± 10	0.05
Exposed to PEO		
PAN/VC + PEO–NH_2 (5k)[c]	32 ± 3	0.05
PAN/VC + PEO–SC (20k)[c]	49 ± 2	0.05
Double-skin membranes		
Unmodified: PAN/VC	164 ± 19	
Derivatized with PEO		
PAN/VC–PEO–NH_2 (5k)	128 ± 59	0.51
PAN/VC–PEO–NH_2 (20k)	121 ± 7	0.05
PAN/VC–PEO–SC (8k)	190 ± 1	0.04
PAN/VC–PEO–SC (20k)	165 ± 14	0.83

[a]The nominal surface area of the fiber was calculated from the length l and the other diameter OD: πl(OD).

[b]Significance is set at $p \leq 0.05$.

[c]Control fiber used in PEO-grafting experiments.

nMWCO curves of percent rejection vs. radius for polydisperse dextran. The rejection coefficient, which provides information on the membrane's sieving properties,[9] did not change with PEO modification of PAN/VC.

Results of the BCA protein adsorption assay, comparing PAN/VC-PEO fibers with those of PAN/VC, are expressed in Table II as amount of protein adsorbed per nominal surface area of the fiber. The exact surface area is indeterminate due to the presence of pores; mercury porosimetry may be useful in determining the total surface area available for protein adsorption. The Mann-Whitney U-statistical test values are included with the data in Table II (p values).

Diffusion coefficients are reported in Table III for the diffusion of α-chymotrypsinogen across three fiber types (all double skin) each measured after storage in either buffer or a 0.4% solution of insulin: (1) unmodified PAN/VC; (2) PEO-grafted PAN/VC; (3) PAN/VC + PEO controls.

Biocompatibility of PAN/VC and PAN/VC–PEO fibers were assessed in terms of their interaction with brain tissue. The fibers were compared as described in Table IV for the response to the implanted hollow fiber membranes

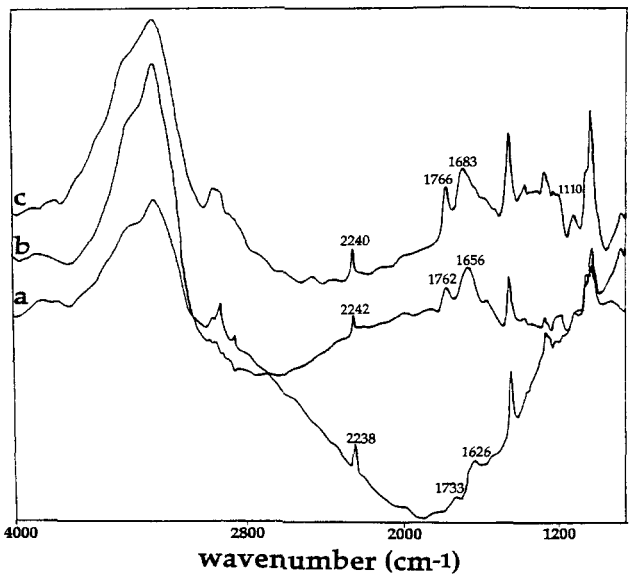


Figure 2. Attenuated total reflectance infrared spectra (absorption vs. wavenumber) of (a) PAN/VC, (b) PAN/VC–CO_2H, and (c) PAN/VC–PEO–NH_2.

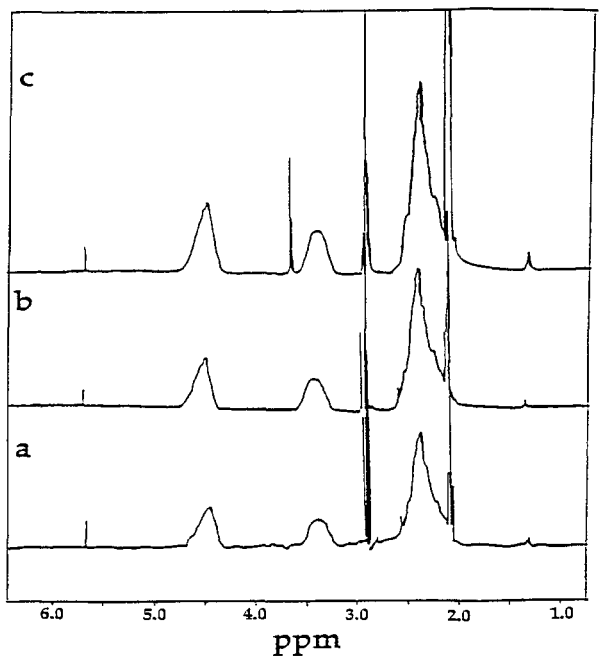


Figure 3. Proton nuclear magnetic resonance spectra (intensity vs. chemical shift) of (a) PAN/VC, (b) PAN/VC–NH_2, and (c) PAN/VC–PEO–NH_2 membranes.

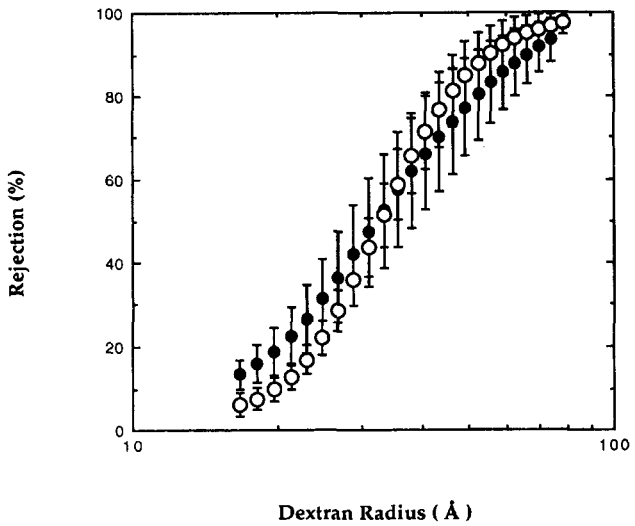


Figure 4. MWCO curves showing the rejection coefficient with polydisperse dextran of (○) PAN/VC and (●) PAN/VC–PEO–NH_2.

of MACs, FBGCs, PMNs, EOs, and reactive astrocytes. Mann-Whitney *U*-test values were calculated and are included in Table IV. The histologic micrographs (H&E and α-GFAP stained), included in Figure 5, represent a fiber with acceptable biocompatibility and an overall rating of 1. Interactions of cell-loaded P(AN/VC) and P(AN/VC)–PEO fibers with the brain tissue were evaluated in the same way as empty fibers and followed the trends described in Table IV.

DISCUSSION

The PAN/VC hollow fiber membranes were modified with derivatized poly(ethylene oxide) in an aqueous solution in which PAN/VC is neither soluble nor swollen. The PAN/VC–water interface is sharp, enhancing a surface selective reaction of PAN/VC with derivatizing reagents to produce PAN/VC–CO_2H and PAN/VC–NH_2. The subsequent reaction between derivatized PAN/VC and end-functionalized PEO is limited by the first step in both depth of reaction and yield. More PEO–SC than PEO–NH_2 is grafted to the derivatized PAN/VC membrane because the conversion of PAN/VC to PAN/VC-NH_2 is more successful than that to PAN/VC–CO_2H, as evidenced by ^1H NMR and as indicated by ATR FTIR spectra. Although extensive surface analysis was not performed, ATR FTIR spectra indicate that the PEO grafting reaction is within the outer micrometer of the surface and is thus surface selective.

The nMWCO (defined as 95% rejection) data in Table I suggest that PAN/VC and PAN/VC–PEO sieve BSA and dextran with similar rejection coefficients. In addition, the rejection curves for polydisperse dextran (Fig. 4) show insignificant differences between PEO-grafted and PAN/VC membranes. Interestingly, the rejections of polydisperse dextran are lower than those of albumin: albumin with a stokes radius of 36.1 Å is rejected at 95% whereas dextran with the same radius is rejected at approximately 60%.

Table III. Comparison of device diffusion coefficients for α-chymotrypsinogen of untreated and insulin-treated PAN/VC–PEO and unmodified PAN/VC double-skin fibers.

Sample	D_m (cm^2/s, $\times 10^8$) (n = 3, ±20%)	D_m (insulin treated) (cm^2/s, $\times 10^8$) (n = 3, ±20%)
PAN/VC	0.3	3.0
PAN/VC–PEO–NH_2 (5k)	0.9	1.0
PAN/VC + PEO–NH_2[a] (5k)	0.4	1.0
PAN/VC–PEO–NH_2 (20k)	1.6	2.2
PAN/VC + PEO–NH_2[a] (20k)	0.9	2.0
PAN/VC–PEO–SC (8k)	0.8	0.7
PAN/VC + PEO–SC[a] (8k)	1.0	1.7
PAN/VC–PEO–SC (20k)	0.6	0.7
PAN/VC + PEO–SC (20k)	0.7	1.3

[a]Control fibers used in PEO grafting reactions.

In sieving through the membrane, albumin adsorption is significant and thereby reduces the pore radius whereas dextran adsorption is insignificant. The hydraulic permeability of the unmodified PAN/VC hollow fiber membrane does not change significantly with modification; however, in some experiments (not tabulated), a larger difference was observed which may be an artifact of handling. The surface modification reaction appears to have little effect on the convective transport properties of the PAN/VC membrane. These data indicate that the pore structure of the PAN/VC membrane is not affected by PEO-grafting; scanning electron micrographs (not shown) also show no change in bulk morphology after PEO grafting.

The data in Table II indicate that BSA adsorption decreases with PEO grafting. The amount of BSA adsorbed is primarily useful as a comparison between unmodified and PEO-grafted PAN/VC because the calculated surface area excludes that of the pores. On the single-skin membranes, the amount of adsorbed BSA to PAN/VC (57 $\mu g/cm^2$) decreases by 50% and 75% for PEO–NH_2 and PEO–SC, respectively ($p \leq 0.05$). The PEO–SC (20,000) grafted PAN/VC shows a greater decrease in BSA adsorption than the PEO–NH_2 (5,000) grafted fiber, indicating that either the modification is more successful with the PEO–SC mechanism or that 20k PEO decreases protein adsorption more effective than 5k PEO. From ^1H NMR results, more PEO–SC (20k) than PEO–NH_2 (20k) is present on PAN/VC. The difference observed in amount of BSA adsorbed to PEO–SC and PE–NH_2 is partially due to the increased amount of PEO–SC grafted; the difference may also result from increased length and mobility of 20k PEO relative to 5k PEO. Desai and Hubbell[8] showed that PEO grafted to poly(ethyleneterephthalate (PET) decreased both protein adsorption and cell growth relative to PET; they further demonstrated that PEO of molecular weight greater than 18,500 g/mol was more effective than lower molecular weight PEO (5k, 10k) in reducing platelet adherence, protein adsorption, and cellular interactions. This effect of increased PEO chain length on reduced protein adsorption was observed by

Table IV. *In vivo biocompatibility rated in terms of host's response level to sealed hollow fiber single-skin membranes.*

Sample (n = 4)	MACs (p values)[a]	FBGCs (p values)[a]	PMNs	EOs
PAN/VC	1.75	2	1	1
PAN/VC–PEO–NH_2 (5k)	1.5 (0.75)[a]	1 (0.05)	1	1
PAN/VC–PEO–SC (20k)	1.25 (0.47)	1.25 (0.16)	1	1
PAN/VC + PEO–NH_2 (5k)	1.25 (0.47)	1.25 (0.16)	1	1

MACs = macrophages; FBGCs = foreign body giant cells; PMNs = polymorphonuclear leukocytes; EOs = eosinophils.

[a]The p values are relative to unmodified PAN/VC using Mann-Whitney U-test; significance is set at $p \leq 0.05$.

Mori et al.,[21] who showed that platelet adhesion decreased for PVC–g-PEO relative to PVC and that adhesion decreased further as the length of the PEO chain increased.

Less BSA adsorbs to PAN/VC–g-PEO than to PAN/VC exposed to PEO (controls for grafting experiment). Since the amount of BSA that adsorbs to PAN/VC–PEO–NH_2 (5k) (grafted) and PAN/VC + NH_2 (5k) (exposed) is the same (within standard deviation), it is reasonable to assume that adsorbed PEO–NH_2 is as effective as grafted PEO–NH_2 at reducing BSA adsorption.

The amount of BSA that adsorbed to double-skin fibers is greater than that which adsorbed to single-skin fibers. In addition, the data for double skin fibers are not as consistent as for single-skin fibers. Although less BSA adsorbs to PEO–NH_2 grafted PAN/VC relative to PAN/VC, no decrease in adsorbed amount of BSA is observed for PEO–SC (8k, 20k) grafted PAN/VC fibers. The p values from the Mann-Whitney U-test confirm that the differences are not significant except between PAN/VC and PAN/VC–PEO–NH_2 (20k). This difference in single-skin vs. double-skin fibers is not fully understood but may reflect the inaccuracy in estimating the surface area available for protein adsorption.

The BSA adsorption results aid in the understanding of diffusion data of α-chymotrypsinogen. As indicated in Table III, diffusion is apparently enhanced for both PEO-grafted PAN/VC and insulin-treated, unmodified PAN/VC relative to control (untreated and unmodified) PAN/VC fibers. (Insulin "treatment" refers to the process of immersing fibers in a 0.4% insulin solution prior to testing). However, diffusion through PAN/VC–PEO is not affected by insulin treatment. Some α-chymotrypsinogen that is permeating through the PAN/VC fiber is likely adsorbed onto the membrane surface, thereby decreasing the measured diffusion coefficient relative to that of PAN/VC–PEO. Since PAN/VC–PEO adsorbs less protein than PAN/VC (Table II), it is affected less by this artifact. In adsorbing to PAN/VC, insulin likely saturates the binding sites and prevents further protein adsorption during diffusion testing. Insulin treatment has little effect on the diffusion coefficient through PAN/VC–PEO membrane devices because PEO effectively reduces protein adsorption which underlies this phenomenon.

In vivo biocompatibility was assessed on hollow fiber membranes after 4 weeks in situ. A true host response could be evaluated after 4 weeks, whereas at 2 weeks, trauma associated with surgery may dominate the host tissue response. Based on the response of reactive cells to the implanted hollow fiber membrane in the central nervous system (CNS) and when compared to Sham surgical controls with a score of 1, PAN/VC shows average biocompatibility with an overall rating of 1.8 (n = 4), whereas PEO-grafted PAN/VC [PAN/VC–PEO–NH_2 (5k and 20k)] shows improved biocompatibility with an overall rating of 1.2 (n = 8). The overall biocompatibility rating was calculated from the responses of MACs, FBGCs, PMNs, and EOs to the implanted hollow fiber membrane, as indicated in Table IV. Using the Mann-Whitney U-test, a statistical difference (p values) between PAN/VC and PEG-grafted PAN/VC existed for the response of FBGCs but not for that of MACs (EOs or PMNs). The in vivo experiment indicates that, qualitatively, the biocompatibility is enhanced for PAN/VC–PEO with respect to PAN/VC. The response of PMNs to the implanted fibers was acceptable (or 1, Table IV), indicating that all implants were sterile and that no gross contamination was introduced during surgery. In addition, eosinophils were not found at the implant site (1, Table IV), indicating that the membrane did not evoke an allergic response.[23] The response of immunoreactive GFAP astrocytes to the different fiber types follows the trends described above. Results from present studies suggest that implants would be well tolerated for at least 1 year as compared to previous results.[24]

CONCLUSION

The PAN/VC hollow fiber membranes were successfully surface modified by a two-step chemical reaction involving conversion of the nitrile to a reactive group with which end-terminated PEO reacted in an aqueous solution. The latter reaction, limited both by the depth and yield of the first reaction, appeared to be surface selective by ATR FTIR spectra. This method allows for prefabrication of PAN/VC membranes with subsequent derivatization in an aqueous solution, which does not affect the transport properties of the material. The transport properties would likely be

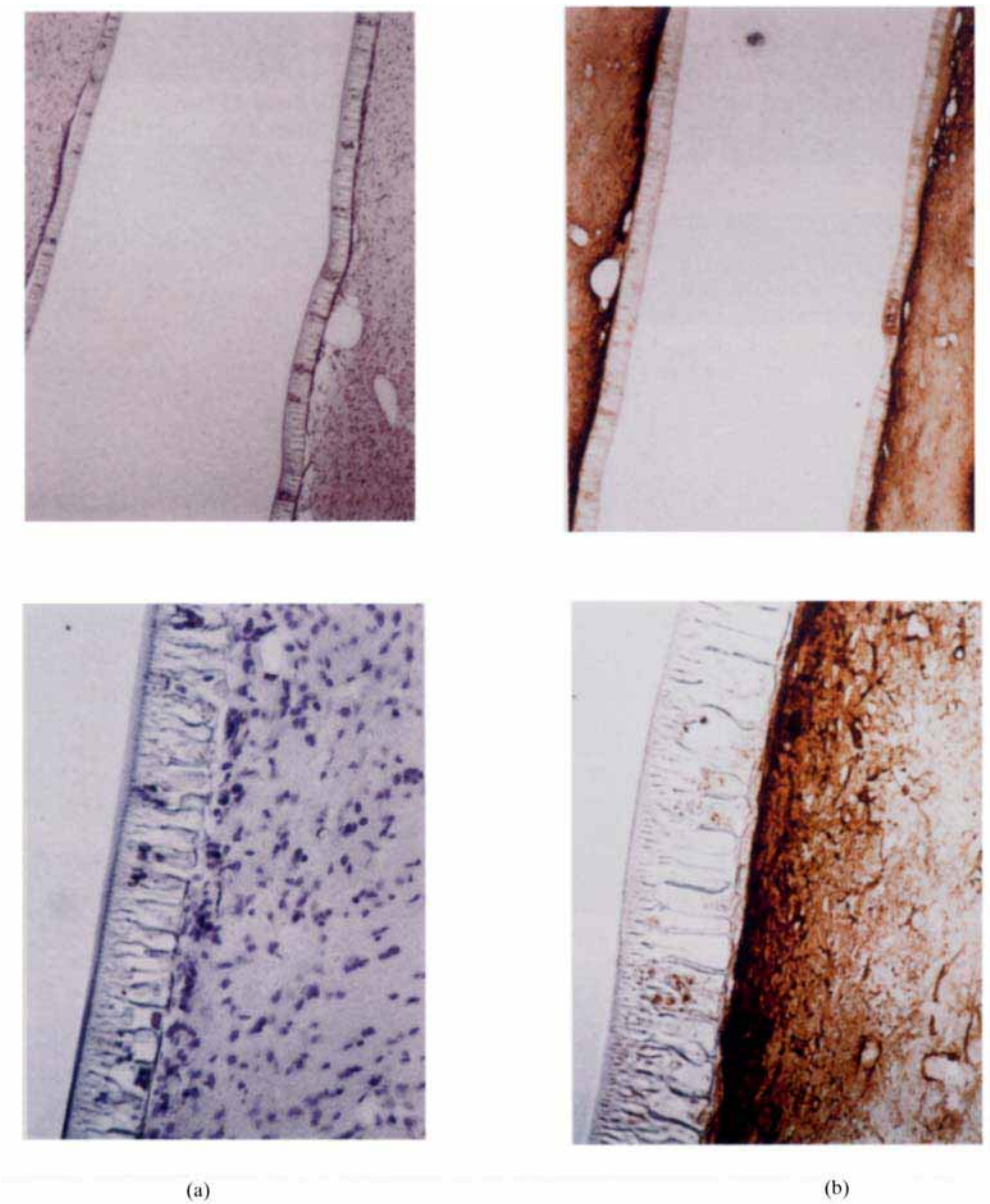


Figure 5. Histologic micrographs of brain fiber sections showing good biocompatibility of PAN/VC–PEO by staining with (a) H&E: (i) ×12.5, (ii) ×62.5; (b) GFAP: (i) ×12.5, (ii) ×62.5.

affected if PAN/PVC were modified with PEO prior to membrane fabrication.

PEO-grafted PAN/VC fibers showed decreased protein adsorption and enhanced diffusion properties with respect to unmodified PAN/VC. The apparent diffusion coefficient of PAN/VC was also enhanced by insulin treatment; however, this is believed to be an artifact of surface protein sorption. Insulin treatment did not affect the measured diffusion coefficient of PAN/VC–PEO. The nominal molecular weight cutoff did not change appreciably after the surface modification reaction with PEO. The molecular weight cutoff data indicate that the pore structure was not adversely affected by the chemistry involved in grafting poly(ethylene oxide). Both PEO–NH_2 control fibers (5k and 20k) had a greater amount of protein adsorbed and a lower diffusion coefficient with respect to PEO–NH_2 derivatized fibers. In vivo biocompatibility results reflect the trends observed in vitro; the in vivo biocompatibility of PAN/VC, although satisfactory, is further enhanced by PEO grafting. Additional studies are currently underway to duplicate the in vivo effects in implants containing living cells. Experiments are planned to determine whether PEO grafting enhances the flexibility and handling of PAN/VC membranes for biohybrid implants.

All animal tests were performed in accordance with written protocols that had been approved by the CytoTherapeutics Inc., Institutional Animal Care and Use Committee.

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