J. Biomater. Sci. Polymer Edn, Vol. 9, No. 7, pp. 713–729 (1998)

Enhancing the interaction of central nervous system neurons with poly(tetrafluoroethylene-*co*-hexafluoropropylene) via a novel surface amine-functionalization reaction followed by peptide modification

YEN W. TONG [1] and MOLLY S. SHOICHET [1,2,*]

[1] *Department of Chemical Engineering and Applied Chemistry,*
[2] *Department of Chemistry, University of Toronto, 200 College Street, Toronto, Ontario, M5S 3E5, Canada*

Received 28 October 1997; accepted 27 January 1998

Abstract—Poly(tetrafluoroethylene-*co*-hexafluoropropylene) (FEP) surfaces were modified with cell adhesive peptides, via a novel amination reaction, to enhance the neuron–substrate interaction. Amination of FEP surfaces was achieved by exposing FEP film samples to a UV-activated mercury/ammonia system for either 3 or 24 h, yielding nitrogen compositions of 3.5 and 13.2%, respectively. By labeling the nitrogen functionality with trichlorobenzaldehyde, the surface amine compositions were calculated to be 14 and 4.3% for the 3 and 24 h amination reactions, respectively. Three oligopeptide sequences derived from laminin (GYIGSR, GRGDS, and SIKVAV) were coupled to the aminated FEP (FEP–NH_2) surfaces and found to have almost identical surface concentrations as determined by XPS. Using radiolabeled GYIGSR, three coupling agents were compared and the concentration of peptide per surface area was calculated to be 3 and 6 fmol cm^{-2} for surfaces aminated for 3 and 24 h, respectively, regardless of the coupling agent. The interaction of embryonic hippocampal neurons with the modified surfaces was compared to that with the positive poly(L-lysine)/laminin control in terms of number and length of extended neurites. After 1 day incubation, neurite extension on the GYIGSR- and SIKVAV-coupled surfaces was similar to that on the positive control but significantly greater than that on FEP and FEP–NH_2 control surfaces. These peptide-coupled fluoropolymer surfaces enhance the neuron–fluoropolymer interaction, similar to that observed with PLL/laminin.

Key words: Fluoropolymer; surface modification; peptides; hippocampal neurons; regeneration; central nervous system.

*To whom correspondence should be addressed. E-mail: molly@ecf.utoronto.ca

INTRODUCTION

Following injury, mature neurons of the central nervous system (CNS) will not regenerate spontaneously, but are capable of regenerating in an environment such as that provided by the peripheral nerve [1–3] or the embryonic CNS [4]. The extracellular matrix (ECM), particularly laminin, mediates axonal elongation during development, providing contact adhesive cues and guidance to axonal growth cones. In an attempt to mimic the ECM found in the peripheral nerve (or during development), a series of laminin adhesive peptides were introduced to the surface of an amine-functionalized fluoropolymer and compared in terms of the response of primary CNS neurons.

Fluoropolymers, such as expanded poly(tetrafluoroethylene) (ePTFE), enjoy widespread use as vascular grafts [5] and dental implants [6]. Fluoropolymers are chemically inert and, consequently, few strategies exist to modify the surface and even fewer methods have been described to introduce nitrogen functionalities. For example, fluoropolymers have been modified by small molecule chemistry [7–9], plasma [10–12], or excimer laser processing [13] to introduce nitrogen functionalities, such as amine [7, 14], hydrazide [15], and nitrile [16]. Recently, it was shown that tertiary fluorocarbons could be aminated using an activated mercury/ammonia system [17]. We take advantage of this mercury/ammonia ('mercat') reaction to introduce amine functionality to the surface of poly(tetrafluoroethylene-*co*-hexafluoropropylene) (FEP) which, unlike ePTFE, has the necessary tertiary carbon for successful amination. The surface amine groups on FEP serve as reactive handles for coupling cell adhesive peptide sequences from laminin, thereby promoting cell interaction with the modified surfaces. The laminin amino acid sequences used to modify FEP-amine surfaces include Arg–Gly–Asp (RGD) [18, 19], Tyr–Iso–Gly–Ser–Arg (YIGSR) [20], and Iso–Lys–Val–Ala–Val (IKVAV) [21]. It has been suggested that the amine functionality alone may act as a cell-adhesion mimic of glycosaminoglycan-binding domains [22, 23]. Previously, FEP that was peptide-modified via a radio frequency glow discharge (RFGD) oxygen functionalization technique [24] required pre-treatment with albumin for an interaction with cell lines to be observed [25]. Herein, the dual effect of amine- and peptide-functionalized surfaces is assessed in terms of the interaction of primary hippocampal neurons in serum-free conditions.

FEP film samples were aminated by vapor phase mercury photosensitization with ammonia [17] for either 3 or 24 h, as shown in Fig. 1. The amine functionality was either quantified by an XPS labeling reaction, using 2,3,5-trichlorobenzaldehyde, or further modified with the laminin adhesive peptides, as shown in Fig. 2. The surfaces were characterized in terms of relative hydrophilicity, atomic composition and chemical functionality. The hippocampal neuron–surface interaction was evaluated qualitatively, in terms of cell adhesion and viability, and quantitatively, in terms of number and length of extended neurites per cell body.

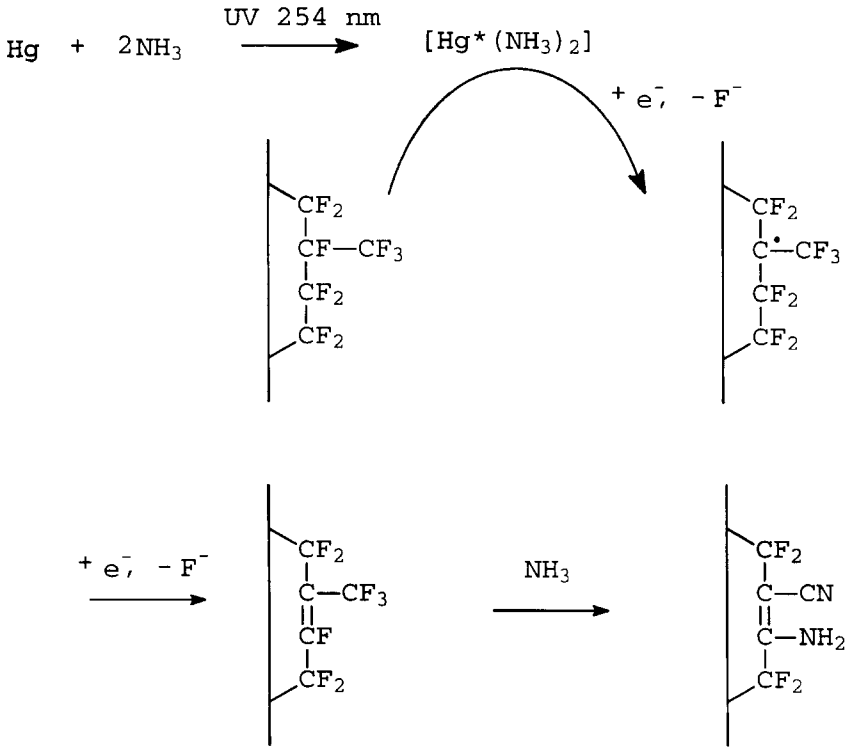


Figure 1. Proposed mechanism for the introduction of amine functional groups to the surface of poly(tetrafluoroethylene-*co*-hexafluoropropylene) (FEP) by a mercury/ammonia UV-photosensitization reaction [17].

EXPERIMENTAL

Materials

All chemicals were purchased from Aldrich (Milwaukee, WI, USA) and used as-received unless otherwise indicated. Tetrahydrofuran (THF, Fisher, Nepean, ON, Canada) was distilled from sodium benzophenone dianion and stored under nitrogen in Schlenk flasks. Hanks balanced salt solution (HBSS), neurobasal medium, B27 supplement and phosphate buffered saline (PBS) were sterile-filtered with 0.22 μm cellulose acetate filters (all from Gibco BRL, Burlington, ON, Canada). All oligopeptides were purchased from Vetrogen, ON, Canada and were used as received. FEP films (5 mm thickness, received from DuPont and cut into 2 × 2 cm samples) were Soxhlet-extracted in THF for 24 h. All water was deionized and distilled from Millipore Milli-RO 10 Plus and Milli-Q UF Plus (Bedford, MA, USA) systems and used at 18 MΩ resistance.

X-ray photoelectron spectroscopy (XPS) data were collected of film samples within 1 week of modification on a Leybold LH Max 200 using a Mg K_α X-ray source at 15 kV and 20 mA emission current. An aperture size of 13 × 7 μm

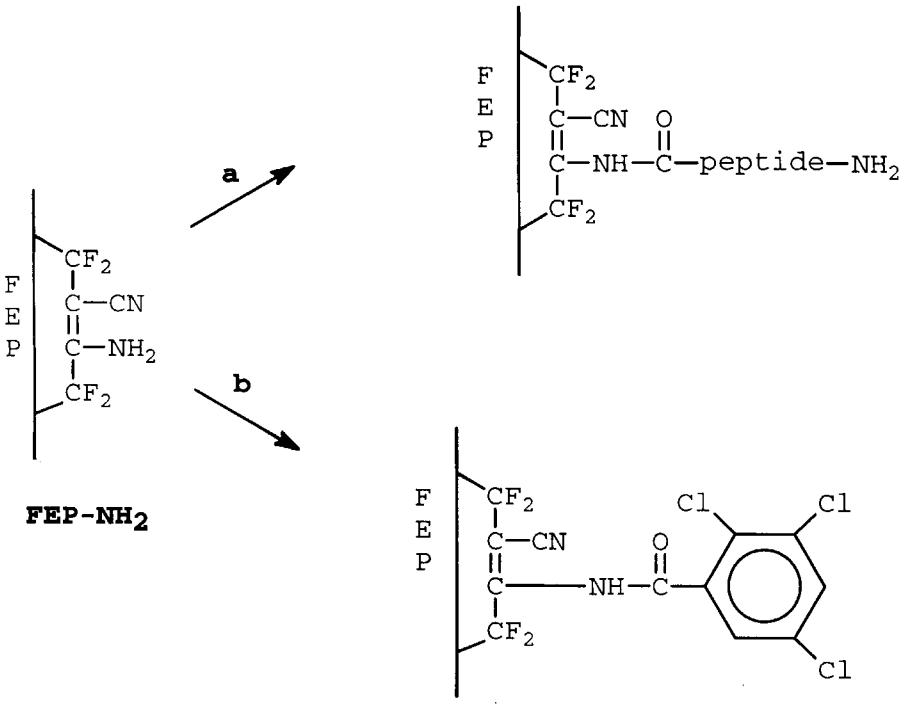


Figure 2. Amine functionalized poly(tetrafluoroethylene-*co*-hexafluoropropylene) (FEP—NH_2) film samples are either (a) coupled with cell adhesive peptides using either tresyl chloride, sulfosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) or *O*-(*N*-succinimidyl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TSU); or (b) labeled with 2,3,5-trichlorobenzaldehyde.

was used to collect data at takeoff angles of 20 and 90 deg between sample and detector ($n = 3$ samples). Unless otherwise specified, all the data presented were taken at a 20-deg takeoff angle. Advancing and receding water contact angles were obtained on a Ramé–Hart NRL telescopic goniometer. Values reported represent the average and standard deviation of five measurements per sample ($n = 3$ samples). Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra of FEP film samples were obtained on a Mattson Galaxy 5400 spectrometer using a germanium crystal (45 deg). Scanning electron microscopy (SEM) micrographs were taken on a Hitachi S4500 field emission microscope at 5 kV acceleration voltage. Iodine-125 radiolabeled tyrosine (Y) of GYIGSR was quantified using a scintillation counter (LKB Wallac 1282-802 Universal γ-Counter) with a 2 × 2 cm sodium iodide detector well of 80% efficiency. Embryonic mouse (CDI Type, Charles River, ON, Canada) hippocampal neurons, that were cultured on all surfaces, were photographed under normal light or filters for fluorescently-stained cells at 20 × magnification under an Axiovert 150 microscope.

Introduction of nitrogen functionality, FEP−NH_2

FEP−NH_2 film samples were prepared using a technique similar to that described by Burdeniuc *et al.* [17]. Briefly, a quartz Schlenk tube, containing FEP film samples and a drop of mercury, was evacuated ($P < 0.01$ mm Hg) and purged with nitrogen (three times). After the fourth evacuation, the tube was re-filled with gaseous ammonia (99.99% purity) to 1 atm pressure and the films were irradiated with eight 15-W mercury lamps (254 nm) in a UV photoreactor (Rayonet) for either 3, 24, or 72 h.

Labeling FEP−NH_2, FEP−NH−CO−$C_6H_2Cl_3$

FEP−NH−CO−$C_6H_2Cl_3$ film samples were prepared by reacting FEP−NH_2 with 20 ml of a 0.012 M 2,3,5-trichlorobenzaldehyde solution in THF for 3 h and then rinsing three times each in THF, methanol, and dichloromethane prior to drying under vacuum. Unmodified FEP film samples were treated identically, thereby, serving as controls.

Peptide coupling to functionalized FEP, FEP−NH−peptide

Three peptides (GYIGSR, SIKVAV, and GRGDS) were coupled to FEP−NH_2 film surfaces using trifluoroethanesulfonyl chloride (tresyl chloride). In addition to tresyl chloride, CGYIGSR was coupled to FEP−NH_2 film surfaces using *O*-(*N*-succinimidyl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TSU) or sulfo-succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), as described below. Unmodified FEP film samples were treated identically and used as controls.

FEP−NH_2 film samples were immersed in a solution containing 200 μl of tresyl chloride in 1 ml pyridine/19 ml THF for 20 min at RT in a Schlenk flask under N_2. Film samples were then added to a beaker containing 10 ml of a 0.2 M pH 10 sodium carbonate-buffered solution and 0.1 $\mu g\,ml^{-1}$ of peptide (i.e. GYIGSR, GRGDS, or SIKVAV) for 24 h [26]. The film samples were rinsed five times each with the buffer solution, dilute hydrochloric acid, water, and THF prior to drying under vacuum.

FEP−NH_2 film samples were immersed in 20 ml of dimethyl formamide (DMF) containing 1.3 mM TSU and 1.3 mM diisopropylethylamine for 4 h at RT in a Schlenk flask under N_2. Film samples were then added to a beaker containing 10 ml of a 0.2 M pH 10 sodium carbonate-buffered solution containing 0.1 $\mu g\,ml^{-1}$ of GYIGSR for 24 h [27]. The film samples were rinsed five times each with the buffer solution, water and THF prior to drying under vacuum.

FEP−NH_2 were immersed in a breaker containing 30 ml of a 0.2 M sodium bicarbonate-buffered solution at pH 10 and containing 2 mg of SMCC. After 4 h, the activated film samples were immersed in a 0.2 M pH 10 buffered sodium bicarbonate solution containing 0.1 $\mu g\,ml^{-1}$ of cysteine-terminated CGYIGSR for 24 h at RT [28]. The film samples were rinsed five times each with the buffer solution, dilute hydrochloric acid, water and THF and then dried under vacuum.

Radioactive labeling of tyrosine

Tyrosine (Y) of GYIGSR was labeled as previously described [27] with radioactive iodine ^{125}I. Briefly, 2 mg of CGYIGSR was dissolved in 5 ml of a pH 11 buffer containing 20 mM sodium phosphate and 0.15 M sodium chloride and then reacted with 1 mCi of carrier-free Na^{125}I (ICN, Costa Mesa, CA, USA) in the presence of Iodobeads (Pierce, Rockford, IL, USA) for 15 min. Free iodide was removed by successive passes through columns packed with anion-exchange resin (Dowex 1-X8, Aldrich). The labeled peptides were coupled to FEP$-$NH$_2$ surfaces as described above. The FEP$-$NH$-$peptide films were also rinsed with 10 mM sodium iodide to desorb any trace ^{125}I before counting by scintillation.

Hippocampal neuron–fluoropolymer interaction

FEP$-$NH$-$peptide films and controls (FEP and FEP$-$NH$_2$) were immersed in 70% ethanol for 1 h and then rinsed four times with sterile distilled water before air-drying. Positive control surfaces were prepared by coating glass coverslips with 1 ml of an aqueous 1 mg ml^{-1} solution of poly(L-lysine) (PLL, Sigma, M_w = 37 000 g mol^{-1}) for 24 h at 37°C and then with 10 μl of a 1 mg ml^{-1} aqueous solution of laminin (Gibco) for 2 h at 37°C. PLL/laminin-coated glass coverslips were rinsed with sterile, distilled water and then air-dried. Embryonic day 18 (E18) mouse hippocampal neurons were isolated, as previously described [29], by dissociation with papain (Worthington Biochemical Corporation) and DNase (Sigma) for 30 min and mechanical trituration in calcium-free HBSS. 1 ml of a hippocampal neuron suspension was then plated at 1 $\times$ 10^6 cells ml^{-1} (or 2.5 $\times$ 10^5 cells cm^{-2}) in serum-free medium (SFM) on each film sample. The SFM consisted of 2 ml B27 supplement, 100 mg chicken egg albumin (Sigma), 10 mg pyruvic acid (Sigma), 1 ml glutamine (Sigma), and 1 ml penicillin/streptomycin (Gibco, 10 000 U ml^{-1}and 10 000 μg ml^{-1}, respectively) in 100 ml of neurobasal medium. The anti-mitotic agent, fluorodeoxyuridine/uridine (Sigma), was added after 12 h. The cells were incubated at 37°C in 5% CO_2 for 4 days. The cell–material interaction was assessed after 1 and 4 days in terms of the number and relative length of extended neurites per cell body (n = 50 cells). Phase contrast micrographs were taken after 1 and 4 days. To assess cell viability at 4 days, samples were incubated for 30 min at 37°C with 150 μl of stock viability assay solution (20 μl ethidium bromide and 5 μl calcein AM in 10 ml PBS, Molecular Probes, Eugene, OR, USA) and examined under fluorescent filters of the optical microscope.

Statistics

Triplicate experimental data were subjected to statistical analysis using SAS (SAS Institute Inc., Cary, NC, USA) on a SGI Challenge L Unix-based computer system. One way ANOVA analysis assuming a constant variance and a 95% confidence interval was used to determine statistical differences of various data sets. Results are

reported as the mean ± the standard error of the mean and statistically significantly different data sets are labeled with different letters.

RESULTS AND DISCUSSION

Introduction of nitrogen functionality, FEP−NH_2

The exposure of FEP film samples to mercury-activated ammonia (i.e. mercat reaction) resulted in the introduction of amine functional groups (FEP−NH_2) as determined by XPS and contact angle. The surface composition of the top 10–40 Å was measured at a takeoff angle of 20 deg while that of the top 40–100 Å was measured at a takeoff angle of 90 deg. In Table 1, the 20-deg takeoff angle data is summarized. After a 3 h exposure, amination was evidenced by a decreased contact angle (84/31 deg) relative to FEP (120/101 deg) and by the presence of nitrogen in the XPS spectrum ($C_{46.2}F_{46.4}O_{3.9}N_{3.5}$). The increased hydrophilicity of FEP−NH_2 relative to FEP is reflected by the lower contact angles and the XPS composition having a higher nitrogen and oxygen and lower fluorine concentrations. After a 24 h exposure, the contact angle was further decreased (27/22 deg) and the nitrogen concentration further increased ($C_{72.5}F_{3.7}O_{10.6}N_{13.2}$), relative to the 3 h exposure, indicating that the extent of amination can be controlled by exposure time. The reaction was self-limiting as amination did not increase at exposure times greater than 24 h. For example, after 72 h of exposure to the mercat reaction, aminated FEP film samples had similar properties to those aminated for only 24 h. After the 72 h exposure, the water contact angles were 31/20 deg and the atomic composition at a 20-deg takeoff angle was $C_{71.4}F_{4.5}O_{11.1}N_{13.0}$. Scanning electron micrographs of FEP, FEP−NH_2-*24h* and FEP−NH_2-*72h* were identical and smooth, having no topographical features and indicating that surface chemistry was modified independently from surface morphology. Due to the similarities observed between FEP−NH_2-*24h* and FEP−NH_2-*72h*, further studies were conducted on FEP aminated for 3 and 24 h only.

In order to determine the surface selectivity of the amination reaction, angle resolved XPS was performed by comparing the 20- and 90-deg takeoff angle data. For a 3 h exposure, FEP−NH_2-*3h* film samples had more nitrogen and less carbon and fluorine at a 20-deg takeoff angle ($C_{46.2}F_{46.4}O_{3.9}N_{3.5}$) than that at a 90-deg takeoff angle ($C_{41.4}F_{53.3}O_{3.1}N_{2.3}$), indicating the surface selectivity of this reaction. For a 24 h exposure, the FEP−NH_2-*24h* film samples had an equivalent amount of fluorine and less nitrogen at the 20-deg takeoff angle ($C_{72.5}F_{3.7}O_{10.6}N_{13.2}$) than that at the 90-deg takeoff angle ($C_{65.2}F_{3.7}O_{10.8}N_{20.4}$), indicating that reactive ammonia penetrated below the FEP surface. Up to 24 h, the longer the exposure time, the deeper the modification. The increased nitrogen concentration at greater depths for FEP−NH_2-*24h* reflects the solubility of ammonia in FEP. By comparing the 20- and 90-deg takeoff angle XPS data, it is clear that the surface of FEP−NH_2-*24h* is enriched with carbon and depleted in nitrogen which may result

Table 1.
Average contact angle and XPS data of surface modified FEP film samples ($n = 3$ samples). XPS data were taken at a takeoff angle of 20 deg between the sample and detector

Film type	Contact angle (θ_A/θ_R)	XPS atomic composition (%)	Contact angle (θ_A/θ_R)	XPS atomic composition (%)
Clean FEP	$120 \pm 2/101 \pm 2$	$C_{31.1}F_{68.9}$	$120 \pm 2/101 \pm 2$	$C_{31.1}F_{68.9}$
	Amination for 3 h		Amination for 24 h	
FEP−NH_2	$84 \pm 2/31 \pm 2$	$C_{46.2}F_{46.4}O_{3.9}N_{3.5}$	$27 \pm 2/22 \pm 1$	$C_{72.5}F_{3.7}O_{10.6}N_{13.2}$
FEP−NH−CO−$C_6H_2Cl_3$	$86 \pm 2/46 \pm 2$	$C_{42.7}F_{49.0}O_{5.2}N_{2.3}Cl_{0.9}$	$66 \pm 3/20 \pm 1$	$C_{62.8}F_{13.2}O_{12.8}N_{10.0}Cl_{1.3}$
FEP + $C_6H_2Cl_3$−CHO (control for labeling)	$119 \pm 2/101 \pm 2$	$C_{32}F_{68}$	$119 \pm 2/101 \pm 2$	$C_{32}F_{68}$
FEP−NH−GYIGSR	$74 \pm 4/21 \pm 1$	$C_{51.8}F_{36.6}O_{7.7}N_{3.9}$	$56 \pm 2/20 \pm 3$	$C_{70.0}F_{6.5}O_{14.0}N_{9.5}$
FEP−NH−GRGDS	$77 \pm 3/22 \pm 2$	$C_{53.3}F_{35.8}O_{7.2}N_{3.7}$	$59 \pm 2/14 \pm 1$	$C_{71.8}F_{4.2}O_{14.7}N_{9.3}$
FEP−NH−SIKVAV	$71 \pm 1/18 \pm 2$	$C_{52.3}F_{36.8}O_{7.0}N_{3.9}$	$67 \pm 3/13 \pm 1$	$C_{69.7}F_{9.5}O_{12.2}N_{8.6}$

from surface rearrangement of the functional groups to minimize surface free energy. While FEP−NH_2-*24h* films were modified to a greater depth of modification than FEP−NH_2-*3h* films, no peaks, other than those ascribed to unmodified FEP, were evident in the ATR-FTIR spectra of all film samples. This indicates that the mercat reaction was limited to depths of modification significantly less than 1 μm (the sampling depth of ATR-FTIR).

The presence of surface oxygen functionality, although not accounted for by the mechanism in Fig. 1, likely results from air-oxidation of either or both the carbon–carbon double bonds or the nitrile group.

Labeling FEP−NH_2, FEP−NH−CO−$C_6H_2Cl_3$

The labeling reaction of FEP−NH_2 with trichlorobenzaldehyde (FEP−NH−CO−$C_6H_2Cl_3$) was used to estimate the concentration of surface primary and secondary amines that would be available for peptide modification. As shown in Table 1, relative to FEP−NH_2 (3 or 24 h), FEP−NH−CO−$C_6H_2Cl_3$ surfaces were less hydrophilic, as expected, due to the phenyl ring. FEP−NH-*3h*−CO−$C_6H_2Cl_3$ surfaces had an increased receding contact angle whereas FEP−NH-*24h*−CO−$C_6H_2Cl_3$ surfaces had an increased advancing contact angle relative to FEP−NH_2-*3h* or FEP−NH_2-*24h*, respectively. The increased hysteresis between advancing and receding contact angles of FEP−NH_2-*24h*−CO−$C_6H_2Cl_3$ film surfaces reflects their chemical heterogeneity relative to that of FEP−NH_2-*3h*−CO−$C_6H_2Cl_3$ films. Since the control reaction of FEP with trichlorobenzaldehyde resulted in FEP (cf. Table 1 for XPS and contact angle data), we can assume that all of the chlorine results from the reaction of trichlorobenzaldehyde and primary and secondary amine groups of FEP−NH_2. Assuming 100% yield for the amine labeling reaction with trichlorobenzaldehyde, the XPS atomic chlorine to nitrogen ratios can be used to calculate the minimum percentage of nitrogen present as primary and secondary amines. For example, for FEP−NH_2-*3h*−CO−$C_6H_2Cl_3$ films, the surface chlorine concentration was 0.9% indicating that 0.3% of the total 2.3% surface nitrogen concentration exists as primary or secondary amines. Thus 14% of the nitrogen functionality of FEP−NH_2-*3h* is available for further modification with peptides. By a similar analysis, FEP−NH_2-*24h*−CO−$C_6H_2Cl_3$ films had 1.3% chlorine and thus 0.43% of the total 10.0% (or 4.3%) surface nitrogen was available for further modification with laminin adhesive peptide sequences. The greater hysteresis in the contact angle data for FEP−NH_2-*24h*−CO−$C_6H_2Cl_3$ is consistent with the greater chemical heterogeneity observed. The relative number of surface amine groups to FEP repeat units was determined from both the 20-deg XPS fluorine to nitrogen data and the calculated amine concentrations. For FEP−NH_2-*3h*, there was 1 amine group per 23 FEP repeat units and for FEP−NH_2-*24h*, there was 1 amine group per 1.6 FEP repeat units.

Since a low percentage of nitrogen functionality was available for further chemical modification with peptides, methods will be developed to increase both their reactivity and the percent of surface amine groups. For example, based on the

mechanism in Fig. 1, the amine groups will be more reactive after reducing the carbon–carbon double bonds to single bonds. To further increase the number of amine groups, the nitrile group will be reduced to primary amine.

Peptide coupling to functionalized FEP, FEP–NH–peptide

FEP–NH_2 surfaces were modified with three cell adhesive peptides (GYIGSR, GRGDS, SIKVAV) using tresyl activation. For the FEP–NH_2-*3h* films, the contact angles decreased after peptide modification reflecting the increased hydrophilic nature of the peptide group on FEP–NH–peptide relative to FEP–NH_2-*3h* (cf. Table 1). The XPS data confirm the contact angle data: FEP–NH–peptide surfaces have depleted fluorine and increased carbon, oxygen and nitrogen. For FEP–NH_2-*24h*, the advancing contact angles increased while the receding contact angles decreased after peptide modification. The XPS data indicate that similar amounts of the three peptides GYIGSR, GRGDS and SIKVAV were introduced to the FEP–NH_2 film surfaces for each amination time (i.e. 3 and 24 h); however, a greater concentration of peptides were introduced to FEP–NH_2-*24h* than to FEP–NH_2-*3h*. The increased hysteresis observed for FEP–NH_2-*24h*–peptide indicates a chemically heterogeneous surface and likely less peptide per nitrogen than that observed for FEP–NH_2-*3h*–peptide as confirmed by the labeling reaction. The XPS data confirm this observation with a surface enrichment of oxygen and fluorine after peptide modification for FEP–NH_2-*24h*. The increased fluorine concentration may reflect surface rearrangement, facilitated by the use of THF that slightly swells FEP.

To determine which of the three coupling agents, tresyl chloride, SMCC and TSU, could yield the greatest amount of peptide per surface area, ^{125}I radiolabeled tyro-

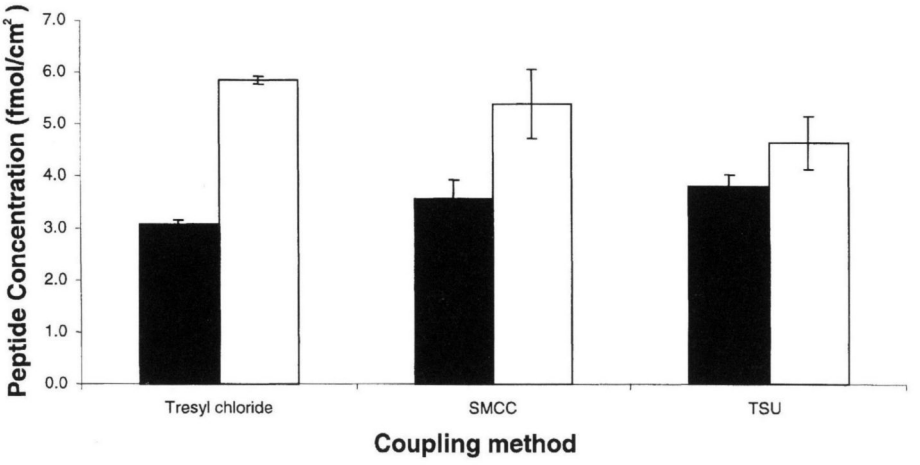


Figure 3. The surface peptide concentrations of FEP–NH–GYIGSR films were determined by counting the γ-radiation of ^{125}I radiolabeled tyrosine (Y) of GYIGSR. The radiolabeled GYIGSR peptide was coupled to FEP–NH_2 using tresyl chloride, SMCC or TSU. The mean with the respective standard error of the mean are plotted for films aminated for 3 (■) and 24 h (□).

sine (Y) of CGYIGSR was coupled to FEP−NH_2 surfaces. As shown in Fig. 3, the amount of peptide that was introduced to FEP−NH_2-*24h* was greater than that introduced to FEP−NH_2-*3h* for all coupling reagents; however, the differences between the three reagents were statistically indistinguishable within 95% confidence. Given the low amount of peptide introduced, the lack of differentiation among the coupling reagents may reflect the low surface concentration of reactive amine groups.

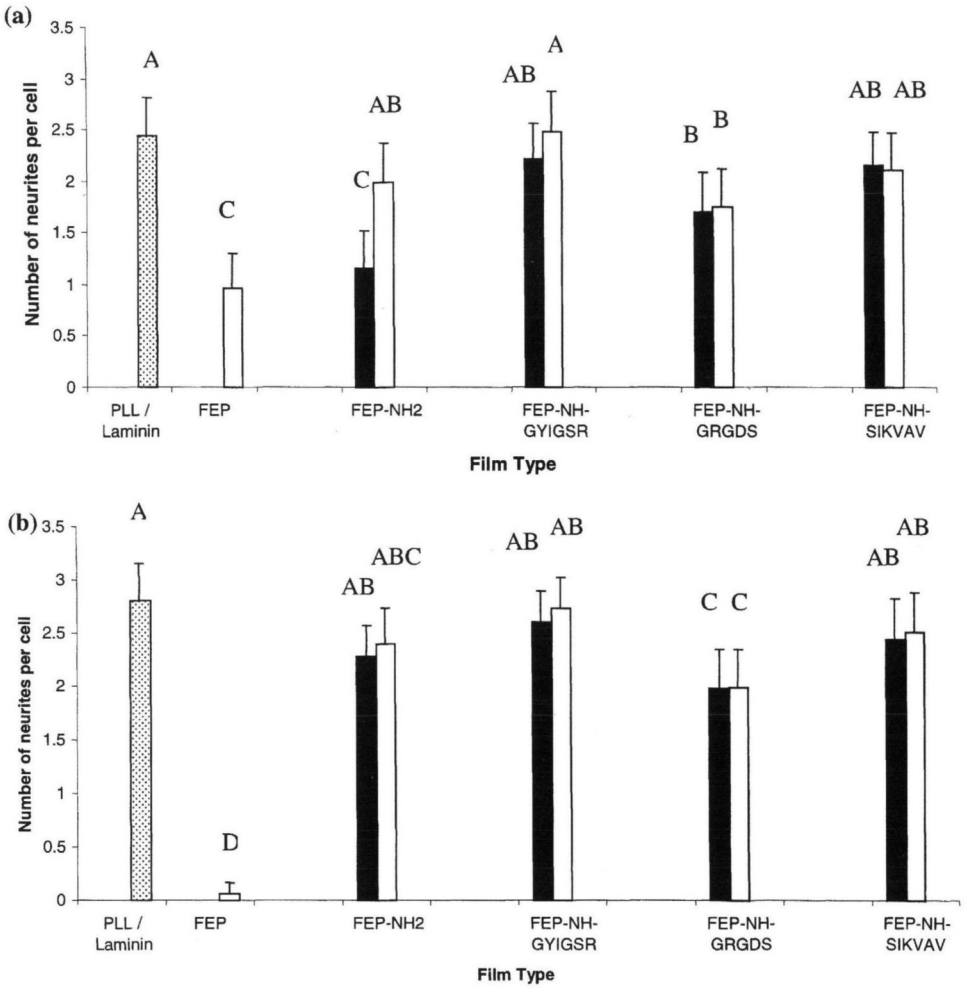


Figure 4. The number of neurites extending per cell (averaged over 50 cells) of embryonic day 18 (E18) hippocampal neurons cultured in serum-free medium on PLL/laminin-coated glass coverslips, FEP and FEP−NH−peptide (peptide = GYIGSR, GRGDS, SIKVAV) surfaces after (a) 1 and (b) 4 days of plating. Surfaces were prepared from FEP−NH_2 for 3 (■) vs 24 h (□). The mean numbers of neurites per cell body with the standard error of the mean are reported for $n = 50$ cells, repeated twice. Data sets with a similar letter are statistically the same whereas those with different letters are statistically different from FEP−NH_2 controls of the same conditions (the data were calculated using one one way ANOVA with $p < 0.05$).

Hippocampal neuron–fluoropolymer interaction

The peptide-functionalized surfaces were compared, in terms of the response of hippocampal neurons, to FEP–NH_2, FEP and a positive control, i.e. glass modified with PLL/laminin. The number of neurites extending per cell body (averaged over 50 cells) is summarized in Fig. 4 after 1 and 4 days of plating. In Fig. 4, statistical differences in the data are designated with different letters. The biggest differences between the surfaces were observed after 1 day of plating. The response observed, from greatest to least, on the FEP–NH_2-*3h* surfaces was FEP–NH-*3h*–GYIGSR ~ PLL/laminin ~ FEP–NH-*3h*–SIKVAV > FEP–NH-*3h*–GRGDS > FEP–NH_2-*3h* ~ FEP. For FEP–NH_2-*24h*, the or-

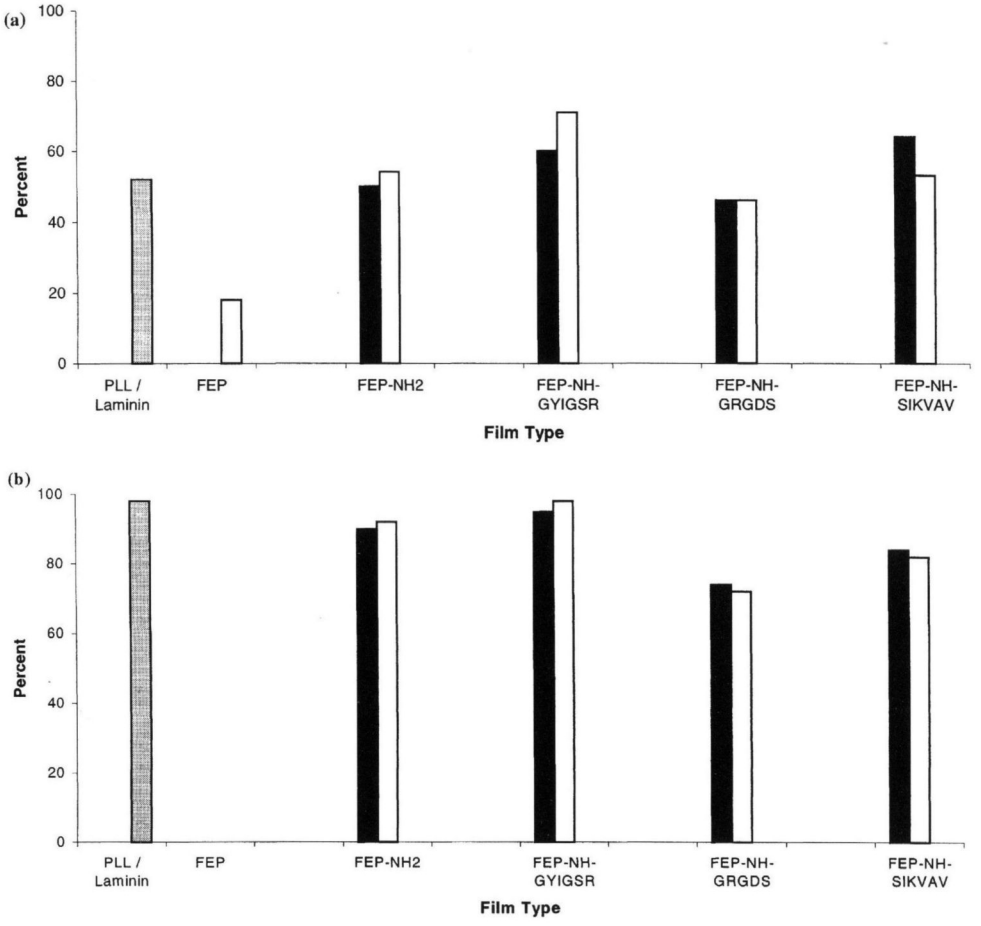
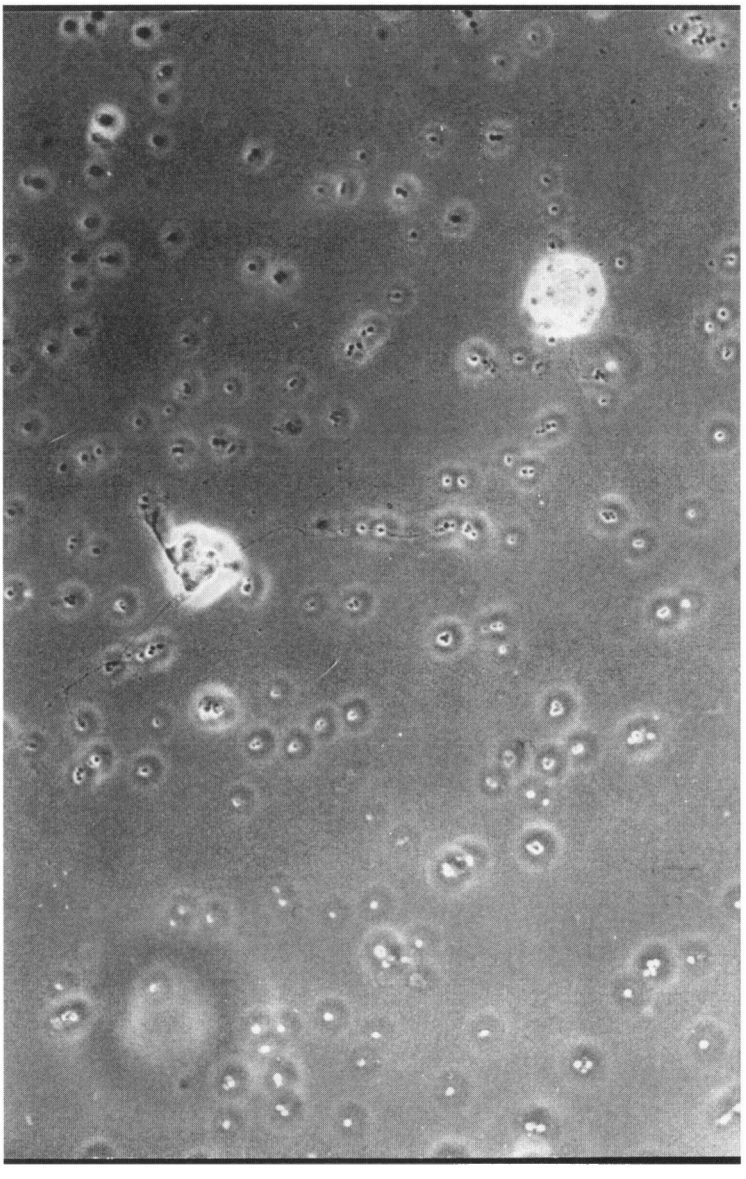


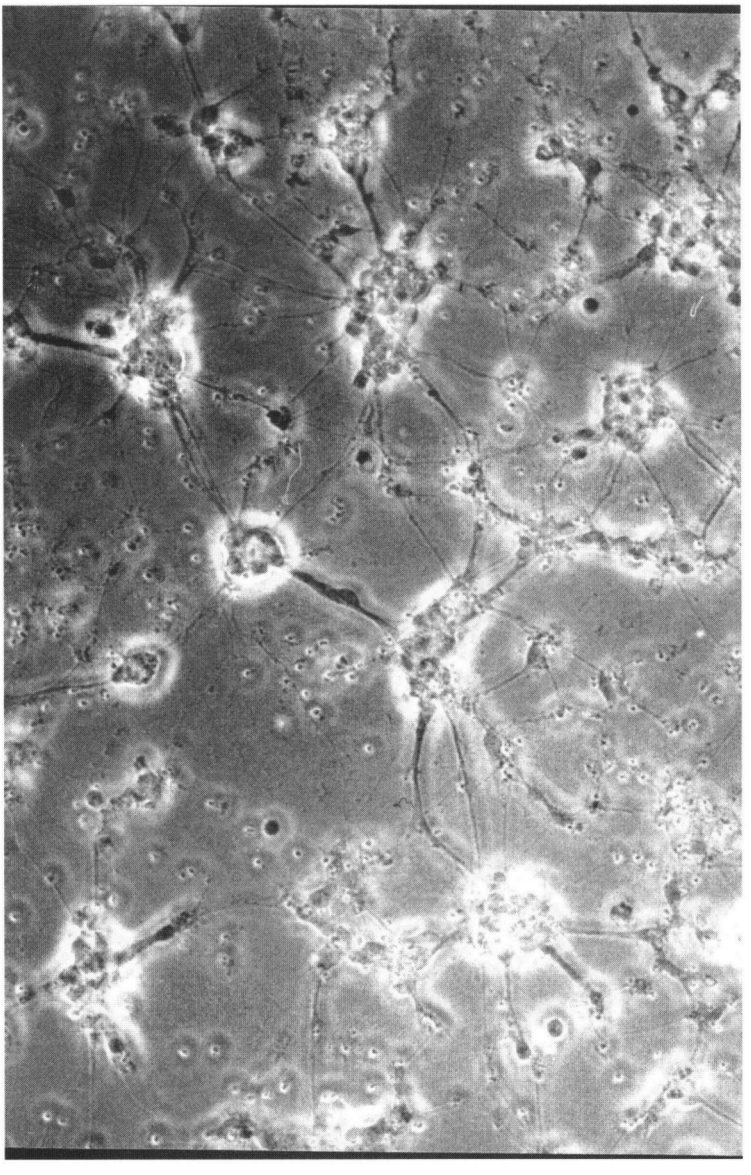
Figure 5. The percentage of cells (n = 50 cells, for two film samples) with neurites longer than one cell body length, of embryonic day 18 (E18) hippocampal neurons cultured in serum-free medium, were compared on PLL/laminin-coated glass coverslips, FEP and FEP–NH–peptide (peptide = GYIGSR, GRGDS, SIKVAV) surfaces after (a) 1 and (b) 4 days of plating. Films were aminated for either 3 h (■) or 24 h (□).



(a)

Figure 6. Phase contrast micrographs of hippocampal neurons plated on different functionalized surfaces after 4 days of incubation demonstrate the enhanced interaction after peptide modification: (a) unmodified FEP, (b) FEP−NH_2-*3h*, (c) FEP−NH−GYIGSR.

der was FEP−NH-*24h*−GYIGSR ~ PLL/laminin > FEP−NH-*24h*−SIKVAV ~ FEP−NH-*24h*−GRGDS ~ FEP−NH_2-*24h* > FEP. After 4 days of plating, the differences among the peptide-functionalized surfaces, FEP−NH_2 and PLL/laminin were difficult to elucidate; however, no cells were supported on FEP. While there was no statistical difference between PLL/laminin controls and GYIGSR- and

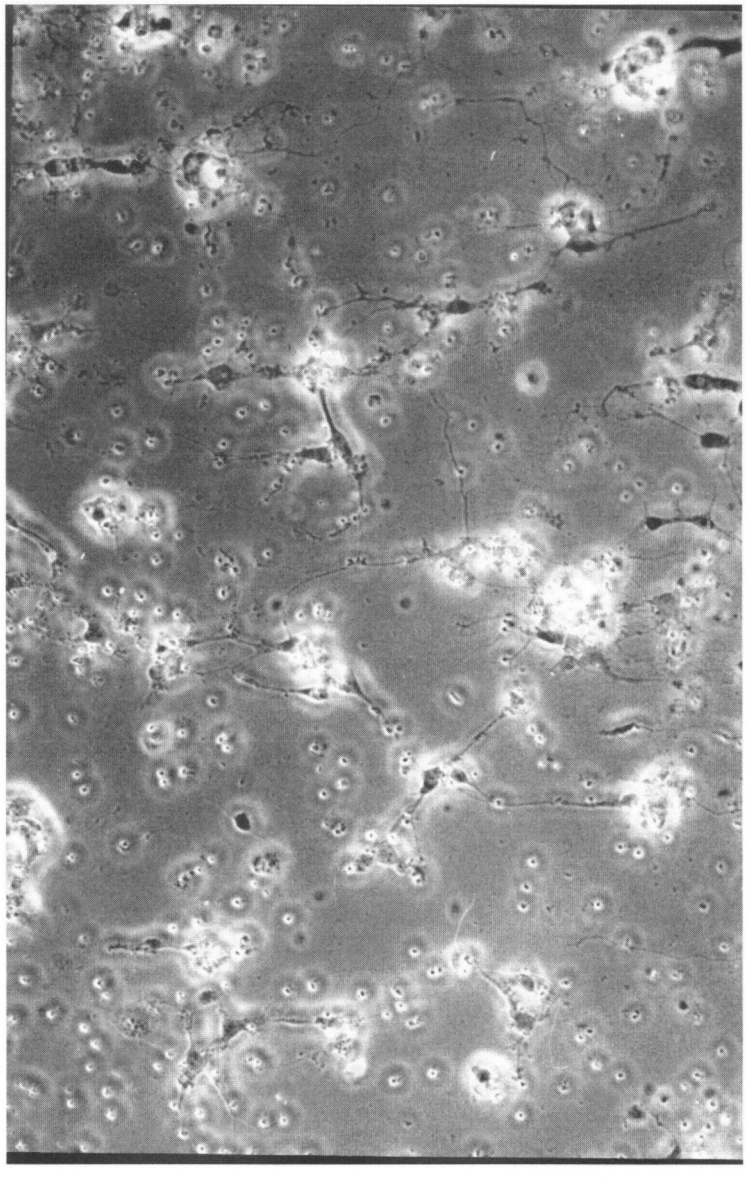


(b)

Figure 6. (Continued).

SIKVAV-functionalized surfaces, PLL/laminin supported statistically more axonal interaction than both the GRGDS- and NH_2-functionalized FEP surfaces.

In order to further gauge the cell-material interaction, the relative length of neurites on the modified surfaces was compared by calculating the percentage of cells having one or more neurites greater than the cell body length, as shown in Fig. 5. After 1 day of plating, the response was greatest on FEP—NH—GYIGSR and least on FEP, with the other surfaces showing similar percentages of cells having



(c)

Figure 6. (Continued).

long neurites between these two extremes. After 4 days of plating, all peptide-modified surfaces had similar percentages, with FEP−GRGDS having the lowest response of the peptide surfaces. By using both number and length of neurites as indicators of the cellular response to the modified surfaces, it is clear that, of the three peptides studied, the GYIGSR-functionalized surfaces are the most conducive to cellular interaction while the GRGDS-functionalized surfaces are the least conducive. These results are consistent with other results we have obtained

using different surface chemistry to achieve peptide immobilization [30]. The phase contrast micrographs, included in Fig. 6, represent the range of responses observed between the hippocampal neurons and the FEP surfaces. FEP supported neither cell adhesion nor neurite outgrowth (cf. Fig. 6a). FEP−NH_2 supported both cell adhesion and neurite outgrowth, yet the large cell clusters indicate that the neurons prefer to grow on each other than on the surface (cf. Fig. 6b). FEP−NH−GYIGSR supported both cell adhesion and neurite outgrowth; the enhanced interaction of neurons with this surface is represented by the distribution of cells (cf. Fig. 6c).

CONCLUSIONS

FEP films were surface-modified with amine functional groups after exposure to the mercat reaction for 3 and 24 h, resulting in 14 and 4.3% of reactive amines, respectively. Despite the lower percentage of nitrogen present as amine groups for the modification at 24 h, more nitrogen was present on FEP−NH_2-*24h* than on FEP−NH_2-*3h*. Coupling the oligopeptide CGYIGSR with FEP−NH_2-*3h* and -*24h* surfaces resulted in surface peptide concentrations of 3 and 6 fmol cm^{-2}, respectively, using tresyl chloride, SMCC or TSU. The hippocampal neuron-material interaction was compared on the following surfaces: nitrogen-functionalized FEP, peptide-coupled films, including GYIGSR-, SIKVAV- and GRGDS-functionalized FEP, unmodified FEP (control) and PLL/laminin-coated glass coverslips (positive control). From the neurite extension and neurite length results, we conclude that the peptide-modified surfaces, and in particular FEP−NH−GYIGSR, enhanced the hippocampal neuron interaction and best mimicked the effects of PLL/laminin surfaces *in vitro*. These surface modification methods may impact strategies used to enhance nerve regeneration.

Acknowledgement

The authors are grateful to the Natural Sciences and Engineering Research Council of Canada and the University of Toronto for financial support. The authors thank Professor Peter Pennefather for supplying the hippocampal neurons and for related discussions.

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