

SPATIAL CONTROL OF NEURONAL CELL ATTACHMENT AND DIFFERENTIATION ON COVALENTLY PATTERNED LAMININ OLIGOPEPTIDE SUBSTRATES

JOHN P. RANIERI,* RAVI BELLAMKONDA,* EVAN J. BEKOS,† JOSEPH A. GARDELLA JR,†
HANS J. MATHIEU,‡ LAURENCE RUIZ‡ and PATRICK AEBISCHER*§

*Division of Surgical Research, Centre Hospitalier Universitaire Vaudois, Lausanne University Medical School, 1011 Lausanne, Switzerland;

†Department of Chemistry, State University of New York at Buffalo, Buffalo, NY 14214-3094, U.S.A.;

‡Department of Material Science, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

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Abstract—The spatial control of neuronal cell attachment and differentiation via specific receptor mediated interactions, may provide an effective means for the *in vitro* reconstruction of neuronal cell architecture. In this study, receptor-specific oligopeptide sequences derived from the extracellular matrix (ECM) molecule laminin, a potent neural cell attachment and differentiation promoter were covalently bound on fluorinated ethylene propylene (FEP) films. The degree of receptor-specific cell attachment and the ability to spatially control neurite outgrowth by covalently patterning the oligopeptide sequences on the FEP film surface were assessed.

FEP films were first chemically activated with a Radio Frequency Glow Discharge (RFGD) process that covalently replaces the surface fluorine atoms with reactive hydroxyl groups. Oligopeptides containing the YIGSR sequence from the B1 chain of laminin and the water soluble oligopeptide containing the IKVAV sequence (CSRARKQAASIKVAVSADR) from the A chain were covalently bound to the hydroxylated FEP films. Electron Spectroscopy for Chemical Analysis (ESCA) verified the covalent attachment of the oligopeptides to the material surface.

The degree of receptor mediated NG108-15 cell attachment on immobilized CDPGYIGSR films was determined using competitive binding media. A 78% reduction in cell attachment was observed on films containing CDPGYIGSR in the cell plating medium. Only a 23% reduction in cell attachment was noted on films plated in medium containing a mock CDPGYIGSK sequence. FEP films immobilized with the IKVAV oligopeptide sequence were shown to mediate PC12 cell attachment and a competitive binding medium also significantly attenuated cell attachment on the immobilized films.

The spatial patterning of these oligopeptide sequences to the FEP surface was shown to localize cell attachment and neurite extension on the patterned pathways. The surrounding unmodified FEP surface was inhibitory in serum containing medium and prevented cellular interactions outside the oligopeptide modifications. The spatial immobilization of laminin oligopeptides on FEP films provides a means to organize the attachment and differentiation of neuronal cells in a receptor-specific manner.

Key words: laminin, IKVAV, YIGSR, selective cell attachment, neuronal cells.

Extracellular matrix (ECM) molecules regulate cell attachment and differentiation via receptor-specific interactions during tissue development, growth and wound healing. Small amino acid fragments of these molecules can mimic some of their functions.^{1,27,29} Fibronectin was first demonstrated to contain an RGD amino acid sequence that can specifically interact with an Integrin class of cell membrane receptors.^{22,23} Receptor binding to these sequences can also initiate second messenger systems within the cell that promote growth, gene expression, cytoskeletal organization and differentiation.^{6,9,21,37}

Laminin, a basement membrane glycoprotein, promotes neuronal cell attachment and differentiation.^{16,19,33} Two particular amino acid fragments of the laminin molecule, YIGSR and IKVAV, recognize a 67¹⁷ and 110 kDa¹³ cell membrane receptor, respectively. The 67 kDa receptor influences cell attachment¹⁰ and the 110 kDa receptor is active in both cell attachment and differentiation.^{14,30}

The presence of inhibitory proteins spatially control the growth and guidance of neuronal processes, for review see Ref. 28. For example, retinal axons in the chick tectum have a strong preference for anterior tectal membranes compared to posterior membranes, and this preference

§To whom all correspondence should be addressed at: Division de Recherche Chirurgicale, Pavillon 3, CHUV, 1011 Lausanne, Switzerland.

is due to inhibitory proteins that can be heat-inactivated within the posterior region.³⁶ The expression and localization of certain ECM components like keratan sulfate⁵ also show inhibitory effects towards neurite outgrowth.

Chemically patterned material surfaces that can spatially control neuronal cell attachment and neurite guidance within defined pathways have been previously developed.^{3,7,15} These systems used photolithographic technology to pattern non-permissive hydrophobic chemistries that prevent cell attachment adjacent to primary amine chemistries that are permissive towards cell attachment. Selective cell attachment on the primary amine pathways was observed in serum containing media with relatively few cells attaching on the adjacent hydrophobic regions. More recently our laboratory has shown that the mechanism for this selective cell attachment response on the primary amine pathways and avoidance of the hydrophobic regions was dependent upon the surface's initial adsorption of the serum protein albumin from cell plating media.²⁴ If albumin was not present, the selective cell attachment response on the amine pathways would not occur, resulting in equivalent attachment on both the hydrophobic and patterned amine surfaces. The different adsorbing conformations albumin assumes on the hydrophobic and amine substituted regions dominates the surface's contrasting non-specific cell attachment properties.²

Material systems using adsorbed extracellular proteins have been also developed to guide neurite outgrowth by patterning permissive protein pathways adjacent to non-permissive proteins. Keratan sulfate and chondroitin sulfate were used as effective neurite outgrowth inhibitors of chick dorsal root ganglion when patterned in an adjacent configuration to permissive laminin pathways.³¹ The patterned denaturation of a fully coated laminin surface was reported to be effective in forming non-adhesive surfaces adjacent to the remaining active laminin pathways and capable of guiding the outgrowth of leech neurons.⁸

The presentation of receptor-specific ECM amino acid fragments on a material's surface may replace a particular function of the ECM. Cell-type specificity can be obtained due to the exclusivity of interaction between a particular cell's receptor and a covalently immobilized oligopeptide surface.¹² Material surfaces covalently immobilized with amino acid molecules that are receptor specific have been developed to mediate neuronal cell attachment and encourage neurite formation. The active laminin oligopeptides containing the YIGSR and the IKVAV sequences have been previously bound by both their C and N terminus and shown to maintain their conformational activity.^{20,25,29}

This study presents a system that allows receptor-specific cell attachment and guidance of extending neuritic processes in a spatially defined manner. A Radio Frequency Glow Discharge (RFGD) process was used to first chemically activate fluorinated ethylene propylene (FEP) films with reactive hydroxyl groups for oligopeptide coupling. Oligopeptides containing the YIGSR and IKVAV sequences were then attached by either their C or N terminus to fully modified films. NG108-15 neuroblastoma cells and PC12 cells derived from a rat pheochromocytoma were plated onto modified FEP films with their receptors either available for binding or competitively blocked with an excess of soluble oligopeptide to assess the degree of receptor-specific cell attachment on the film surfaces. FEP films were also pattern modified with the above oligopeptides to spatially control cell attachment and direct neurite extension on the film surface.

EXPERIMENTAL PROCEDURES

Surface activation of FEP films

FEP films were a generous gift from Dupont (Wilmington, DE) in 8.5×11 inch sheets, 50 μm thick. The films were cut into discs with a diameter of 3 cm. The discs were chemically modified using an RFGD process that replaces the surface fluorine atoms with covalently bound hydroxyl groups with no discernible change to the surface morphology.³⁸ Fully modified films were chemically activated for 2 min. The patterned film modifications used nickel grids with rectangular openings 300 μm wide by 1500 μm long with a land spacing of 500 μm , or 20 μm wide by 1500 μm long with a land spacing of 100 μm between each opening. The grid was placed directly on top of the film prior to introduction into the modification chamber. The RFGD process was then activated

for 10–15 sec. The modified films were then removed and ultrasonically cleaned in both hexane and methanol for 1 min, placed between clean tissue paper and wrapped in aluminum foil for storage.

ESCA analysis of the N-terminus CDPGYIGSR coupling reaction

ESCA analysis was performed in an ion pumped system with a concentric hemispherical analyzer (Perkin–Elmer, model PHI 5500, PE software multi-technique PHI v.3.0B). Base pressure of the system was below 5×10^{-10} Torr. For ESCA, a twin anode X-ray source was operated at 15 kV and 350 W using Mg Ka radiation. Charge compensation was applied with a standard low voltage electron emission source. Samples were placed on an horizontal sample stage with five degrees of freedom (x , y , z , rotation, tilt). Two take-off angles of the photoelectrons were used (20 and 80° with respect to the surface) for angle resolved ESCA measurements with a precision of $\pm 4^\circ$. For this multi-technique system, energy distribution, energy resolution and analysis area are a function of the analyzer which was controlled by an HP-Apollo computer work station. Spatial resolution of the analyzer was 2 mm up using an electronic lens (Omnifocus II[®]) at the analyzer entrance. Pass energy of the analyzer was set at 23.5 eV giving an energy resolution of 0.5 eV. Instrument calibration was performed with the help of elemental standards such as sputter cleaned Au, Ag and Cu with C_{1s} reference energy at 285.0 eV.

Oligopeptide immobilization reactions on the activated FEP films

(a) *Oligopeptide immobilization on fully modified FEP films.* The oligopeptides GGGGYIGSR, CDPGYIGSR, CDPGYIGSK and CSRARKQAASIKVAVSADR (ANAWA Trading SA, Wangen, Switzerland) were attached by their N-terminus to fully modified FEP hydroxylated films using carbonyldiimidazole (CDI) as a coupling agent. The FEP films were first placed in a glass vessel to which 20 ml of dry tetrahydrofuran (THF) containing 70 mg of CDI was added. The reaction was allowed to proceed for 30 min after which the films were removed and washed thoroughly in a cascade of dry THF. The films were then placed in a glass beaker to which 20 ml of 100 mM sodium bicarbonate buffer was added (pH 8.4) containing a reaction excess of the desired oligopeptide (4 ± 1 mg). The reaction was allowed to proceed for 72 hr at 4°C. The films were then removed and washed in deionized water and successive methanol washes. The oligopeptide sequence YIGSRGGGG was immobilized on the hydroxylated films using a standard nucleophilic substitution reaction (S_N2) that attaches the C-terminus of the oligopeptide to the reactive hydroxyl functionalities of the modified FEP films.²⁵ The YIGSRGGGG oligopeptide was bound by its C-terminus to compare its cell attachment activity to the N-terminus bound configuration. Briefly, 20 ml of reagent grade dimethyl sulfoxide (DMSO) was added to a glass well containing three films and a sample coupon for reaction verification. 4 ± 1 mg of YIGSRGGGG was then added to the DMSO along with 10 mg of potassium carbonate as an acid acceptor. The vessel containing the films was placed on a hot plate and heated between 45 and 50°C. The reaction was allowed to proceed for 72 hr. The films were then removed and ultrasonically washed in deionized water and three successive methanol washes. Sample coupons for both the N- and C-terminus reactions were analyzed with (ESCA) to verify the successful immobilization of the oligopeptide sequences.

(b) *Oligopeptide immobilization on pattern modified FEP films.* To determine the ability of the FEP films to spatially control cell attachment and differentiation, the 19 mer IKVAV containing oligopeptide was immobilized by its C-terminus on 300 μm wide hydroxyl pathways using the protocol mentioned in (a). The CDPGYIGSR sequence was immobilized by its N-terminus to FEP films pattern modified with 20 μm hydroxyl substituted pathways. The same CDI reaction mentioned above in (a) was used with one modification, the reaction time for CDI activation was reduced to 15 min.

Twenty-four hours prior to performing the cell attachment assays, the films were cleaned in a 70% ethanol bath for 15 min and mounted into custom designed 5 ml capacity wells that were sterilized at 70°C for 24 hr. The wells were then placed in sterile 100 mm² Falcon dishes.

Cell culture

NG108-15 cells (a generous gift from Dr M. Nirenberg, National Institutes of Health) were cultured in Dulbecco's modified Eagle's medium (DMEM) and supplemented with 0.1 mM hypoxanthine, 0.4 mM aminopterin, 16 mM thymidine, and 10% fetal calf serum. PC12 cells (ATCC

CRL 1721) were cultured on rat tail collagen-coated polystyrene 100 mm² dishes (Fischer Scientific) in RPMI 1640 (GIBCO), 10% fetal bovine serum, 5% horse serum, 50 ng/ml NGF, and 100 U/ml penicillin–streptomycin. The cells were maintained in tissue culture flasks within an incubator at 37°C in a humidified atmosphere of 94% air and 6% CO₂. Both cell types were mechanically removed from their tissue culture flasks, centrifuged at 250 *g* and then resuspended in the desired cell plating medium.

Cell attachment assays on fully modified FEP films

Three fully modified oligopeptide immobilized films were used for each of the cell attachment assays. The films were first pre-adsorbed with 2 ml of a 0.1% albumin solution in phosphate buffered saline (PBS) at 37°C for 2 hr to block non-specific cell attachment on the FEP surface. The albumin solution was then removed and the films were washed three times with PBS to elute any non-adsorbed protein. Twenty thousand cells for each well were then placed in either 2 ml of serum-free medium or 2 ml of serum-free medium containing a concentration of 1 mg/ml of the desired oligopeptide sequence. After 30 min, the cells were then plated onto the films and placed in an incubator set at 37°C in a humidified atmosphere and 6% CO₂ for 1 hr. Cell attachment was then assessed by sampling in a step-wise fashion on the film surface. A minimum of 300 cells per well under 200X Hoffman optics on a Zeiss Axiovert 100 TV were counted. The Student's *t*-test was used to assess statistical significance ($P < 0.05$) for all attachment assays.

Cell attachment to pattern modified FEP films

Three pattern immobilized films were used for the PC12 and NG108-15 cell attachment assays. Five thousand NG108-15 cells were plated on the 20 μm CDPGYIGSR immobilized films and 75 × 10³ PC12 cells were plated on the 300 μm 19 mer IKVAV immobilized films in 2 ml of serum containing medium. The PC12 cell medium was supplemented with 50 ng/ml of NGF. The wells were then placed within a humidified incubator set at 37°C and 6% CO₂. The selective attachment response and neurite outgrowth on the modified regions was assessed at 8 hr, and one day intervals for up to three days.

RESULTS

Verification of the N-terminus oligopeptide immobilization reaction

ESCA measurements permitted reaction verification on the hydroxylated FEP films. Figure 1 shows the C_{1s} and N_{1s} multiplexes of unmodified FEP, after OH fixation by the RFGD process, after CDI reaction, and after the CDPGYIGSR oligopeptide sequence fixation. Table 1 indicates the expected binding energies from literature^{4,26,35,38} together with the respective experimental values obtained.

ESCA measurements on unmodified FEP shows no surface nitrogen present prior to modification (N_{1s} multiplex). The fixation of hydroxyl groups by the RFGD process leads to a shape change of the C_{1s} FEP+OH multiplex compared to unmodified FEP. Peak deconvolution displays two new bonds that correspond to C–C and C–OH bonds.

The CDI surface modification can be viewed according to the scheme in Fig. 2(A). The N_{1s} FEP+CDI multiplex shows the presence of a nitrogen peak introduced after the CDI reaction (Fig. 1). New bonds such as N–C, N=C are displayed. Concurrently, the C_{1s} multiplex shows the new C=O and C–N bonds. At this stage of the modification, the nitrogen content at the surface was measured to be 1.4%.

The oligopeptide fixation step to the CDI reacted films can be viewed according to scheme in Fig. 2(B). ESCA measurements on the oligopeptide modified samples (FEP+CDPGYIGSR) demonstrated no new bonds created but displayed an increase in the signal intensity of the N_{1s} multiplex corresponding to an increase in the nitrogen content through the oligopeptide sequence fixation. The nitrogen surface content increased to 2.5%. This corresponds to an increased signal intensity of the C–N and C–C oligopeptide skeleton peaks (Fig. 1).

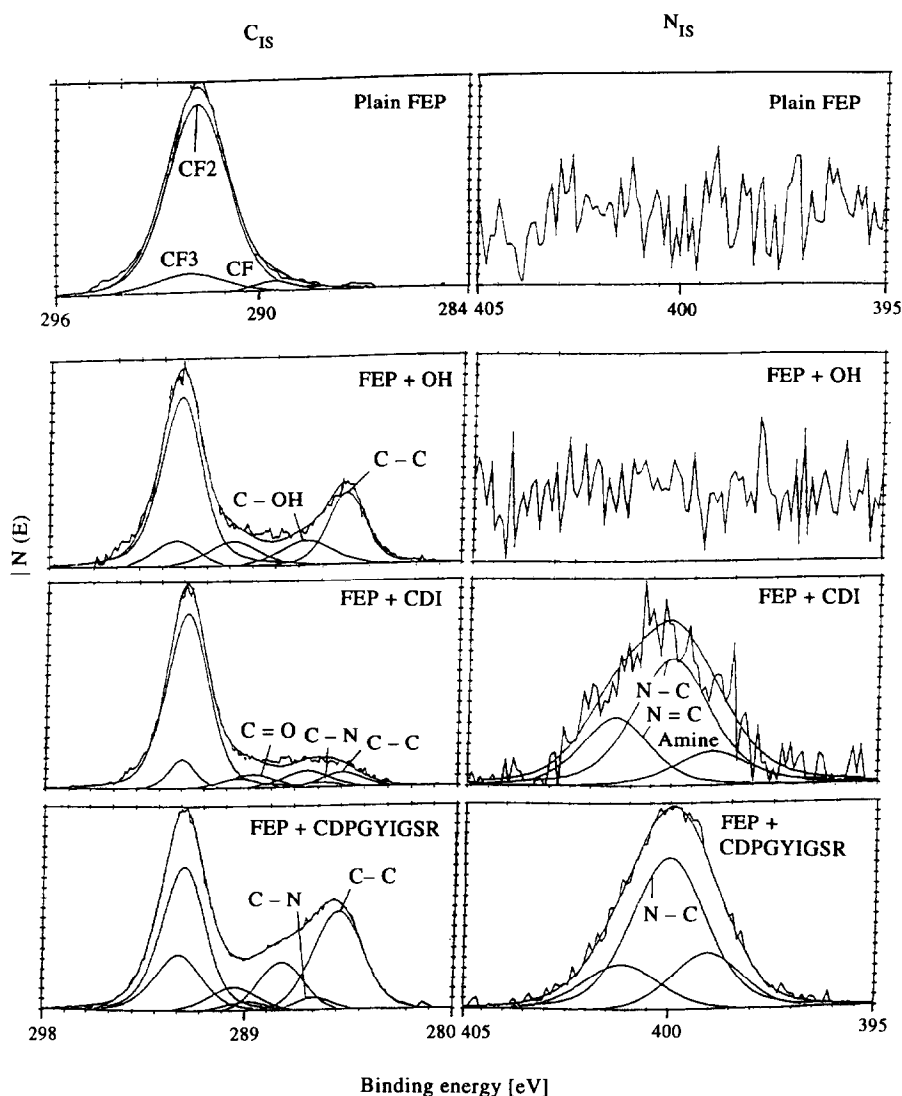


Fig. 1. C_{1s} and N_{1s} spectra of unmodified FEP, after OH fixation by the RFGD process, after the carbonyldiimidazole reaction and after the CDPGYIGSR sequence fixation. The binding energy scale of the C_{1s} plain FEP multiplex differs from the other C_{1s} multiplexes. Spectra were corrected for satellite peaks and sample charging. All bands are referenced to C_{1s} 285.0 eV and N_{1s} 400.0 eV.

Table 1. Expected and measured energy values for the N-terminus coupling reaction to the FEP film surface. Reference values for C_{1s} (285.0 eV) and N_{1s} (400.0) are italicised

Element	Bonds	Energy values expected E_b (eV)	Energy experimental values $E_b \pm 0.1$ eV			
			Plain FEP	FEP+OH	FEP+CDI	FEP+YIGSR
C_{1s}	C-F ₃	292.0	292.0	292.4	292.0	292.0
	C-F ₂	291.8	291.8	292.2	291.8	291.8
	C-F	289.5	289.6	289.9	289.5	289.5
	C=O	288.9	/	/	288.9	288.9
	C-OH	286.4	/	286.7	286.5	287.4
	C-N	285.9	/	/	285.9	286.0
	C-C	285.0	/	285.0	285.0	285.0
N_{1s}	N=C	401.1	/	/	401.4	401.2
	N-C	400.0	/	/	400.0	400.0
	Amine	399.0	/	/	399.0	399.1

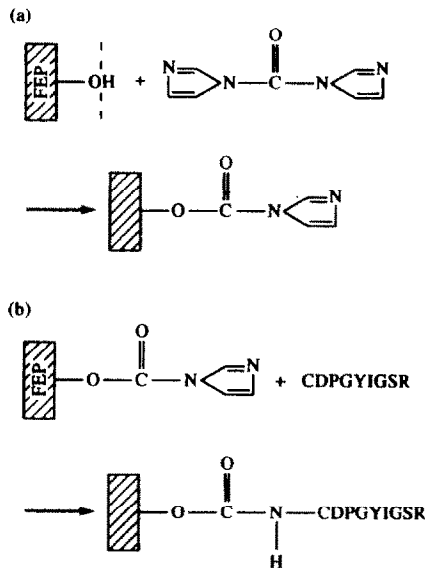


Fig. 2. Chemical representation showing (A) the CDI activation step of the hydroxylated FEP surface; (B) the oligopeptide immobilization reaction to the CDI activated FEP surface.

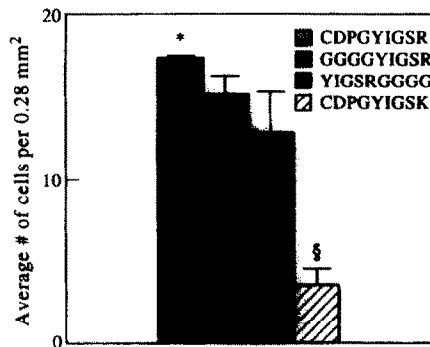


Fig. 3. Histogram showing average NG108-15 cell attachment on FEP films covalently immobilized with the oligopeptides GGGGYIGSR, CDPGYIGSR, and CDPGYIGSK by their N-terminus and YIGSRGGGG by its C-terminus 1 hr after cell plating in serum-free medium. The CDPGYIGSR immobilized films mediated a significantly higher degree of cell attachment compared to the other three oligopeptide immobilized films, $*P < 0.05$. Also, the three active YIGSR containing oligopeptides mediated a significantly higher number of attached cells compared to the mock oligopeptide sequence, CDPGYIGSK, $§P < 0.001$.

NG108-15 cell attachment on the YIGSR immobilized oligopeptide films

NG108-15 cell attachment on N-terminus bound CDPGYIGSR GGGGYIGSR, CDPGYIGSK, and C-terminus bound YIGSRGGGG films in serum-free medium 1 hr after plating is shown in Fig. 3. Each cell attachment assay comprises the average of three individual oligopeptide immobilized films. Average cell attachment on the native CDPGYIGSR sequence was determined to be significantly greater than average cell attachment on either the N-terminus bound GGGGYIGSR or the C-terminus bound YIGSRGGGG sequences ($P < 0.05$). The average cell attachment of NG108-15 cells on the three active oligopeptide sequences were all significantly greater than average cell attachment on the mock sequence, CDPGYIGSK ($P < 0.001$).

Competitive cell attachment assays on CDPGYIGSR and the 19 mer IKVAV containing oligopeptide film surfaces

NG108-15 cell attachment assays on CDPGYIGSR films in serum-free medium containing the soluble oligopeptide sequence CDPGYIGSR or the mock sequence CDPGYIGSK is shown in Fig. 4. A 78% reduction in average cell attachment occurred on the films containing the active oligopeptide in the cell plating medium as compared to cell attachment on CDPGYIGSR films in

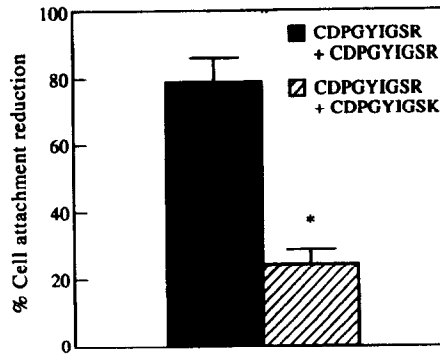


Fig. 4. Histogram showing the percentage of NG108-15 cell attachment attenuation on FEP films immobilized with CDPGYIGSR following a 1 hr exposure to medium containing either soluble CDPGYIGSR or CDPGYIGSK. A significant reduction in cell attachment on films plated in medium containing the active oligopeptide sequence CDPGYIGSR was observed compared to cell attachment in medium containing the mock sequence CDPGYIGSK, $*P < 0.001$.

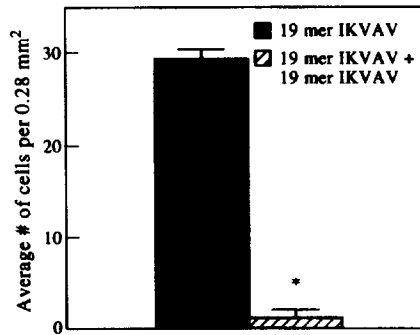


Fig. 5. Histogram showing average cell attachment on FEP films immobilized with the 19 mer IKVAV sequence in serum-free medium and medium containing the oligopeptide 1 hr after plating. Cell attachment on the films plated in medium containing the oligopeptide demonstrated a significant attenuation of cell attachment on the FEP films, $*P < 0.001$.

serum-free medium alone. Comparatively, cell attachment on CDPGYIGSR films plated in serum-free medium containing the mock oligopeptide CDPGYIGSK reduced cell attachment by only 23%. The cell attachment reduction between the two assays was statistically significant ($P < 0.001$).

PC12 cell attachment on 19 mer IKVAV immobilized FEP films in serum-free medium and serum-free medium containing the soluble 19 mer IKVAV sequence is shown in Fig. 5. Cell attachment on the films in the oligopeptide contained medium was significantly attenuated compared to cell attachment in serum-free medium alone ($P < 0.001$).

Cell attachment and differentiation on patterned oligopeptide films

PC12 cells were plated on 300 μm patterned IKVAV substituted FEP films to determine the cell attachment and neurite extension properties on spatially modified substrates. Selective cell attachment on the 300 μm oligopeptide pathways was observed after 8 hr. The cells remained exclusively attached on the oligopeptide pathways after three days in culture. Figure 6(A) shows confluent PC12 cell attachment on the 300 μm oligopeptide pathways after two days. No significant cell attachment was noted on the unmodified FEP regions. Figure 6(B) shows neurite outgrowth from PC12 cells on the 300 μm pathways after the addition of NGF. After two days, the neurites approached the boundary between the oligopeptide modified surface and unmodified FEP regions but were effectively deterred from crossing the interface.

The ability to support selective cell attachment and differentiation on 20 μm pattern modified CDPGYIGSR pathways was evaluated for NG108-15 cells. Figure 7(A) shows the selective attachment response of the NG108-15 cells on the 20 μm pathway modifications after 8 hr in culture. Figure 7(B) shows NG108-15 cell differentiation after one day in culture. Neurite outgrowth

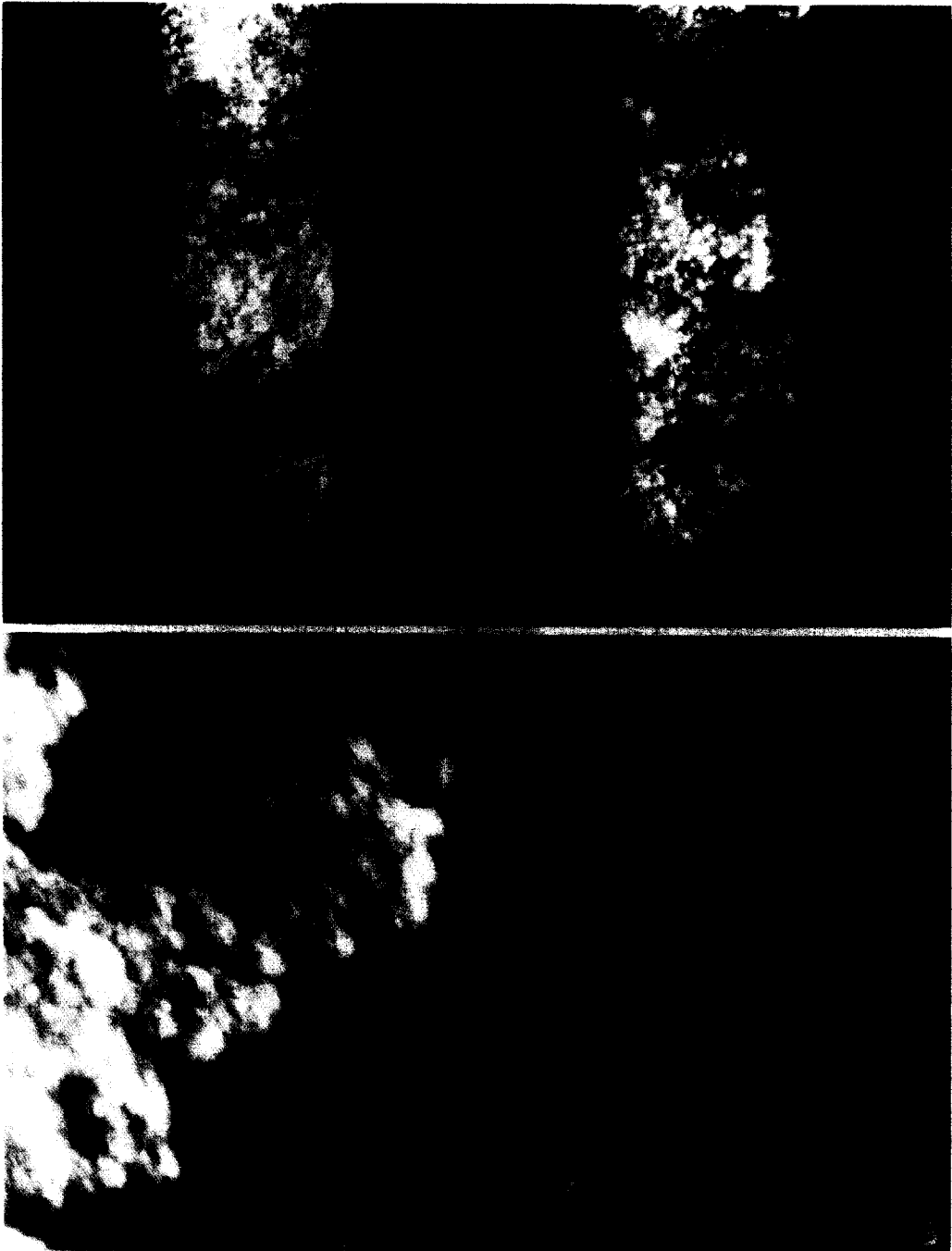


Fig. 6. Micrograph of PC12 cells plated on FEP films pattern modified with the 19 mer IKVAV oligopeptide showing that (A) cell attachment occurs almost exclusively on the $300\ \mu\text{m}$ oligopeptide pathways after two days. Scale bar= $100\ \mu\text{m}$. (B) Neurite outgrowth from PC12 cells after the addition of NGF remain on the modified pathways and avoid the interface of the surrounding unmodified FEP surface after two days in culture. Scale bar= $25\ \mu\text{m}$.

followed the pattern modified oligopeptide pathways with no significant crossover on the unmodified FEP regions observed after three days in culture.

DISCUSSION

The present study shows that an FEP surface covalently immobilized with oligopeptides containing the laminin derived YIGSR and IKVAV sequences, can mediate receptor-specific cell

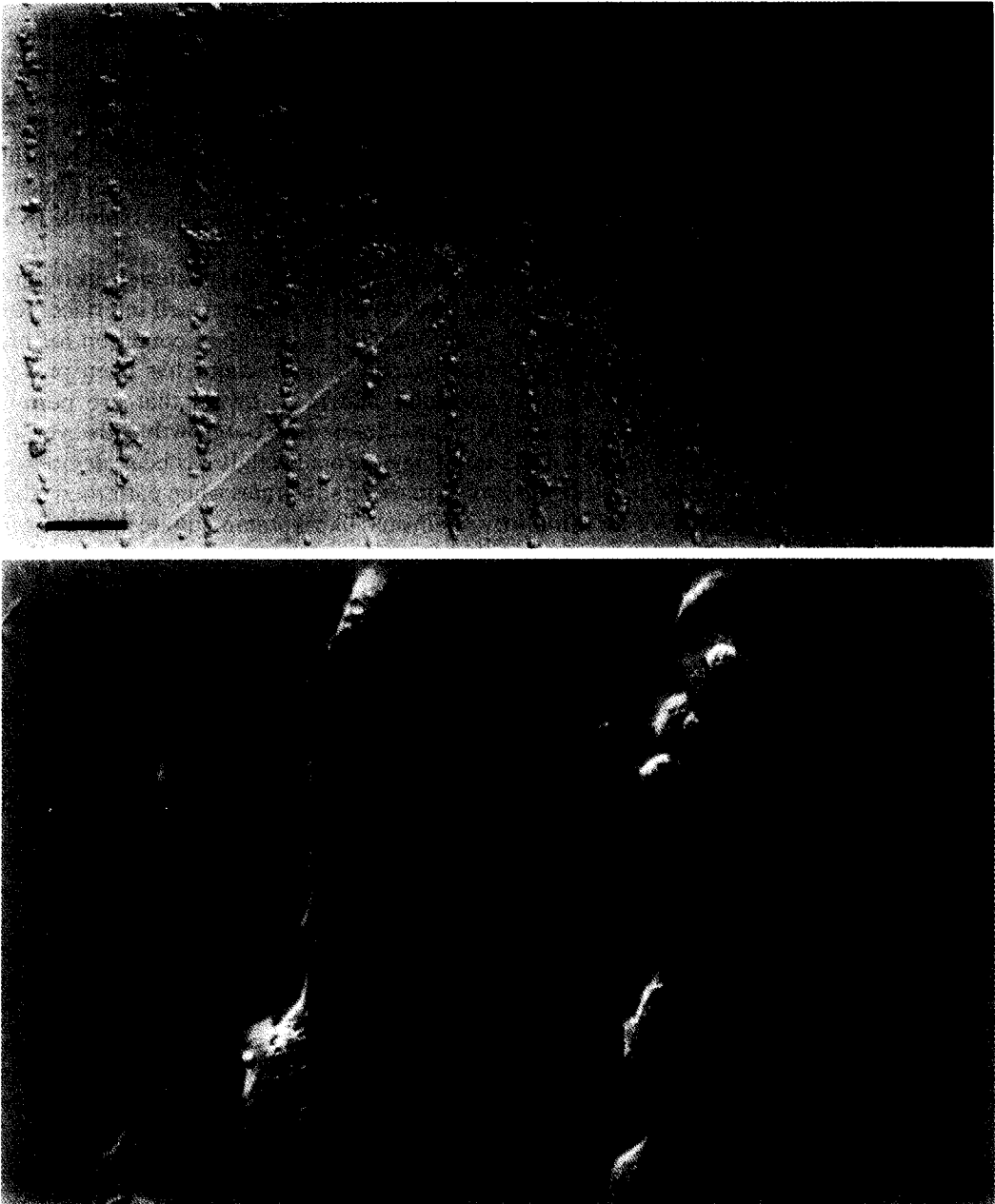


Fig. 7. NG108-15 cells plated on FEP films pattern modified with 20 μm CDPGYIGSR pathways showing after 8 hr that (A) cell attachment occurs almost exclusively on the oligopeptide immobilized pathways. Scale bar=100 μm . (B) After 1 day, neurite outgrowth remains within the oligopeptide immobilized pathways. Scale bar=20 μm .

attachment for NG108-15 and PC12 cells, respectively. Neuronal cell attachment and neurite outgrowth can also be spatially controlled on the FEP films by pattern immobilizing the YIGSR and IKVAV sequences to their surface. The surrounding unmodified FEP regions adjacent to the oligopeptide pathways, serve as an effective non-permissive surface in deterring cell attachment and neurite extension outside the patterned modifications.

The covalent coupling of the oligopeptides by their N-terminus to the FEP surface with CDI chemistry was analytically verified using ESCA analysis. The initial attachment step of CDI to the FEP surface showed a significant increase for the presence of nitrogen on the surface. Curve fitting of the nitrogen peaks also suggests the presence of the expected N–C and N=C bonds. ESCA analysis after oligopeptide coupling to the CDI activated surface shows a further increase in the nitrogen peak. Curve fitting further suggests the presence of the expected covalent C–N linkage between the oligopeptide and FEP surface.¹¹

The most effective YIGSR sequence in promoting cell attachment on the FEP surface was determined to be the native nine amino acid sequence, CDPGYIGSR. The degree of receptor-specific cell attachment on the CDPGYIGSR films was estimated using competitive binding assays containing either soluble CDPGYIGSR or CDPGYIGSK in the cell plating medium. The covalent immobilization of the 19 mer IKVAV containing oligopeptide to the FEP surface by its N-terminus was shown to mediate the attachment of PC12 cells on the FEP film surface. PC12 cell attachment could also be significantly reduced on the films if the cell plating medium contained soluble IKVAV oligopeptide.

A material surface that can spatially control neuronal cell attachment and guide neurite outgrowth requires the molecular control of cell receptor interactions at the material–cell interface. Receptor-specific interactions or “active” adhesion is believed to be a necessary condition for proper cell differentiation.³⁴ The ability to effectively manage these interactions has been previously accomplished by geometrically patterning extracellular matrix pathways that are permissive towards a particular cellular process, for example laminin for neurite outgrowth. However, this is not sufficient to control the guidance of a differentiating neuronal cell. It has been demonstrated that neurites show little selectivity between naturally occurring substrates when patterned adjacent to one another.¹⁸ The presence of an inhibitory chemistry like keratan sulfate whose negative charge may play a role in its inhibitory nature is believed necessary to restrict neurite outgrowth on patterned permissive pathways.³¹

The control of cell attachment on a material surface requires patterned surface chemistries that can effectively manipulate the conformation of adsorbing serum proteins. The type of adsorbed proteins and the particular conformation they assume on a chemically modified surface will then determine the permissive or non-permissive nature of a surface towards cellular interactions. Several material systems have used a permissive primary amine chemistry patterned adjacent to a non-permissive hydrophobic surface to obtain a selective cell attachment response.^{7,15} It has been shown that the mechanism for the selective cell attachment response on an amine substituted surface was the contrasting manner in which albumin pre-adsorbs on the two surfaces. On a hydrophobic chemistry, albumin adsorbs in a non-permissive conformation towards cell attachment. Whereas, albumin adsorbs on an amine substituted surface in larger quantities, but assumes a permissive conformation towards cell attachment.²⁴

In this study, PC12 cells were plated on FEP films covalently patterned with the 19 mer IKVAV containing oligopeptide to determine if selective cell attachment and directed neurite outgrowth would occur along the receptor specific pathways. Almost exclusive cell attachment occurred on the patterned pathways and neurite outgrowth was also spatially restricted to the pathway modifications. NG108-15 cells previously shown to recognize the CDPGYIGSR sequence of laminin also selectively attached and differentiated on the immobilized CDPGYIGSR pathways. Although the inhibitory mechanism of adsorbed albumin on unmodified FEP is passive, it provided a sufficient spatial guidance signal for restricting neurite outgrowth to the patterned oligopeptide pathways.

The ability to pattern modify FEP films with oligopeptide chemistries provides a useful tool for studying the molecular interactions of neural cell attachment and differentiation. The laminin oligopeptide immobilized films in this study were shown to mediate receptor specific cell interactions and the native state of FEP provided an effective negative surface and maintained extending neurites within the spatially immobilized oligopeptide pathways.

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