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# Selective adhesion of astrocytes to surfaces modified with immobilized peptides

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**Abstract**

Under serum-free conditions, rat skin fibroblasts, but not cortical astrocytes, selectively adhered to glass surfaces modified with the integrin-ligand peptide RGDS. In contrast, astrocytes, but not fibroblasts, exhibited enhanced adhesion onto substrates modified with KHIFSDDSSE, a peptide that mimics a homophilic binding domain of neural cell adhesion molecule (NCAM). Astrocyte and fibroblast adhesion onto substrates modified with the integrin ligands IKVAV and YIGSR as well as the control peptides RDGS and SEDSDKFISH were similar to that observed on aminophase glass (reference substrate). This study is the first to demonstrate the use of immobilized KHIFSDDSSE in selectively modulating astrocyte and fibroblast adhesion on material surfaces, potentially leading to materials that promote specific functions of cells involved in the response(s) of central nervous system tissues to injury. This information could be incorporated into novel biomaterials designed to improve the long-term performance of the next generation of neural prostheses. 

*Keywords:* Astrocytes; Neural cell adhesion molecule; Peptide-modified Biomaterials; Cell adhesion; Fibroblasts

## 1. Introduction

Long-term biocompatibility of prostheses intended for the central nervous system may be largely dependent on modulation of gliosis, a tissue-level response to injury that involves the activation of various types of cells including astrocytes and fibroblasts [1,2]; this gliotic response results in encapsulation and isolation of implants [3]. Since establishment of electrical connections between a neural prosthesis and neurons (which initiate, process, and respond to electrical activity in neural tissues) is essential for implant device function, control over the interactions of astroglial cells and fibroblasts with implant surfaces (in order to avoid encapsulation and isolation) may be critical for optimizing the long-term performance of implanted devices in the central nervous system.

Chemical modification of material surfaces with immobilized, bioactive peptides is a promising strategy for directing and controlling cell–biomaterial interactions with a degree of selectivity not possible through other surface modification techniques [4–7]. In the present study, model substrates were modified with select, bioactive peptides including a series of integrin ligands and a novel peptide that mimics a bioactive domain of neural cell adhesion molecule (NCAM, a cell–cell adhesion molecule of the immunoglobulin superfamily of proteins). Astrocyte and fibroblast adhesion (an early cell–substrate response which is critical for subsequent cell function) to these peptide-modified surfaces was examined in order to identify bioactive peptides that, when immobilized onto biomaterial surfaces, may improve the performance of prostheses in the central nervous system.

## 2. Materials and methods

### 2.1. Substrate surface modification

Borosilicate glass coverslips (18 mm in diameter; Fisher Scientific, Albany, NY) were modified with immobilized, synthetic peptides following previously described techniques [5,8] (Fig. 1). Briefly, aminophase

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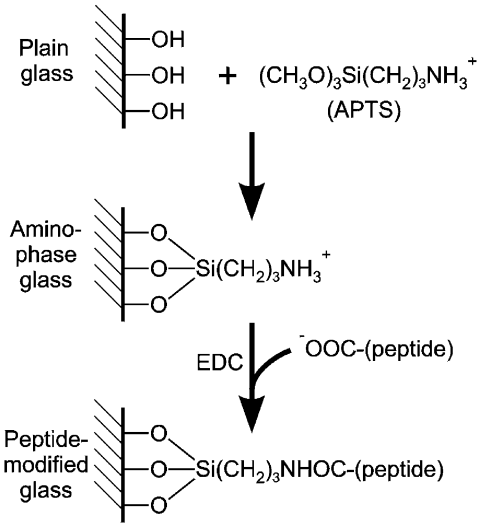


Fig. 1. Outline of the peptide-immobilization process. Synthetic peptides were covalently immobilized onto amine-derivatized glass substrates, or "aminophase" glass, using a carbodiimide-based reaction; EDC = 1-Ethyl-3-(3-dimethylaminopropyl)carboiimide.

substrates were prepared by modifying chemically etched coverslips with aminopropyltriethoxysilane (APTS; Sigma, St. Louis, MO). In order to determine the distribution of immobilized amine groups, randomly selected aminophase glass substrates were stained using the Atto-TAG FQ amine-labeling kit (Molecular Probes, Eugene, OR) following manufacturer instructions and then visualized by fluorescence microscopy.

Synthetic peptides were covalently linked to these amine-modified substrates using a carbodiimide-based reaction [5,8]. The peptides examined in the present study included the integrin-ligand sequences isoleucine-lysine-valine-alanine-valine (IKVAV), arginine-glycine-aspartate-serine (RGDS), and tyrosine-isoleucine-glycine-serine-arginine (YIGSR) as well as a sequence representing a homophilic binding domain of the cell–cell adhesion protein neural cell adhesion molecule (NCAM), lysine-histidine-isoleucine-phenylalanine-serine-aspartate-aspartate-serine-serine-glutamate (KHIFSDDSSE) [9]. Sequence control peptides, which were defined by random rearrangement of the amino acids in the peptides RGDS and KHIFSDDSSE, included arginine-aspartate-glycine-serine (RDGS) and serine-glutamate-aspartate-serine-aspartate-lysine-phenylalanine-isoleucine-serine-histidine (SEDSDKFISH).

Prior to use in assays of cell adhesion, all peptide-modified substrates, as well as the reference substrates of either plain or aminophase glass, were sterilized by brief immersion in 70% ethanol, then attached to the bottom of 22 mm diameter wells of a 12-well tissue culture plate.

### 2.2. Cell cultures

Primary cultures of rat cortical astrocytes were prepared from 3-day old Wistar rat pups following established techniques [10] and maintained in Dulbecco's modified Eagle medium (DMEM), supplemented with 5% fetal bovine serum and antibiotics, under standard cell culture conditions (at 37°C, humidified, 5% $CO_2$/95% air environment). Astrocytes, identified by expression of glial fibrillary acidic protein, comprised greater than 90% of the cells in these primary cultures and were used in cell adhesion assays during the 14–21 day period after isolation.

Rat skin fibroblasts (cell line CRL-1213; American Type Culture Collection) were cultured in DMEM supplemented with 10% fetal bovine serum. Fibroblasts passage 4–27 were used in the adhesion experiments.

### 2.3. Cell adhesion experiments

Either rat cerebral astrocytes ($1.0 \times 10^5$ cells per well) or rat skin fibroblasts ($7.0 \times 10^4$ cells per well) in DMEM without serum were seeded separately onto each peptide-modified and reference (i.e., plain and aminophase glass) substrate, and allowed to adhere under standard cell culture conditions for either 6 (for astrocytes) or 4 (for fibroblasts) h. Adherent cells were fixed in situ with 4% paraformaldehyde (at 37°C) for 20 min and stained with 0.5% cresyl violet (Sigma) for 30 min. Cell adhesion density (cells/mm$^2$) on each substrate was calculated from the average number of adherent cells counted in five randomly selected, $1 \times 1$ mm$^2$ fields per substrate. The cell adhesion experiments were run in duplicate at three separate times. These data were analyzed for statistical significance using Duncan's multiple range test with a protection level of $\alpha = 0.01$.

## 3. Results

### 3.1. Surface characterization of aminophase glass

The process of immobilizing synthetic peptides used in the present study required pretreatment of the substrate surfaces with the aminosilane APTS. This process resulted in a bimodal distribution of amines on substrate surfaces: specifically, staining of aminophase glass with an amine-reactive label revealed a random dispersion of many punctate foci (one such example is indicated by the arrow in Fig. 2A), each measuring 2–3 μm in width; deposited against a diffuse, lower-level density of immobilized amines (Fig. 2A).

Bimodal distributions of peptides on peptide-modified substrates have been reported in the literature [11]. The results of the present study suggest that the arrangement

of immobilized peptides may be a consequence of the distribution of amines on the aminophase surface to which the peptides are grafted. While peptide-modification of substrates using methods similar to those used in the present study typically results in immobilization of pico- to nanomolar quantities per square centimeter [4,5,8,11], the localized regions of high ligand concentration (i.e., the foci of immobilized peptides) may mediate specific events (such as clustering of integrin receptors on the cell membrane into focal contacts) influencing subsequent cellular responses.

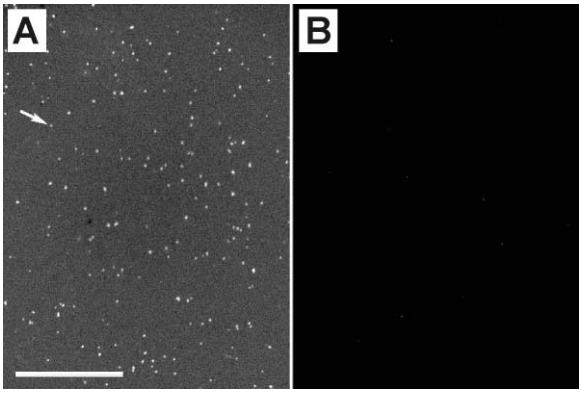


Fig. 2. Surface analysis of aminophase glass. Representative fluorescence micrographs illustrating the distribution of immobilized amines on aminophase glass (A) and on unmodified glass (B). The arrow in Frame A points to a locus of immobilized amines. Scale bar = 50 μm.

### 3.2. Selective adhesion of astrocytes and fibroblasts to surfaces modified with immobilized peptides

Rat skin fibroblasts exhibited maximal, selective adhesion onto aminophase glass modified with the integrin-ligand RGDS (Fig. 3A). Recognition of, and response to, RGDS was sequence-specific since fibroblast adhesion to surfaces modified with RDGS, a non-adhesive or control peptide, was similar to that observed on the reference substrate of aminophase glass (Fig. 3A). Fibroblast adhesion onto surfaces modified with either IKVAV, YIGSR, KHIFSDDSSE, or SEDSDKFISH was also similar to that obtained on aminophase glass (reference substrate), providing evidence that none of these peptides promoted fibroblast

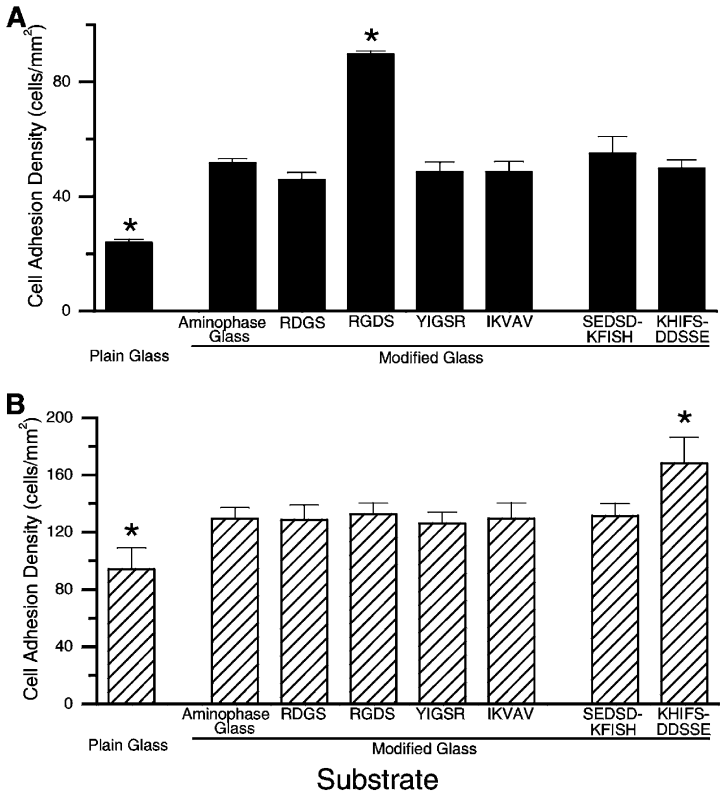


Fig. 3. Selective adhesion of cells to surfaces modified with immobilized peptides. Fibroblasts (A) and astrocytes (B) in serum-free media were allowed to adhere (for 6 and 4 h, respectively), under standard tissue culture conditions onto unmodified glass and aminophase glass (reference substrates) as well as onto glass modified with one of the following select peptides: RGDS = arginine-glycine-aspartate-serine; RDGS = arginine-aspartate-glycine-serine; YIGSR = tyrosine-isoleucine-glycine-serine-arginine; IKVAV = isoleucine-lysine-valine-alanine-valine; KHIFSDDSSE = lysine-histidine-isoleucine-phenylalanine-serine-aspartate-aspartate-serine-serine-glutamate; SEDSDKFISH = serine-glutamate-aspartate-serine-aspartate-lysine-phenylalanine-isoleucine-serine-histidine. For data of astrocyte adhesion, $n = 6$, except for adhesion on SEDSDKFISH for which $n = 3$. For data of fibroblast adhesion, $n = 4$, except for adhesion on either RDGS or SEDSDKFISH for which $n = 3$. Each adhesion experiment was run on duplicate substrate samples. Values are mean ± SEM; *$\alpha < 0.01$ (compared to adhesion results on aminophase glass; Duncan's test).

adhesion (Fig. 3A). The observed selective adhesion of fibroblasts to material surfaces modified with immobilized RGDS, but not with IKVAV, is consistent with results reported in the literature [12].

Rat cortical astrocytes exhibited a unique pattern of adhesion onto the peptide-modified substrates tested in the present study. Adhesion of astrocytes onto aminophase glass modified with the integrin-ligand peptides RGDS, IKVAV, or YIGSR was similar to that observed on aminophase glass, the reference substrate (Fig. 3B). In contrast, astrocyte adhesion was enhanced (compared to that on aminophase glass) on substrates modified with KHIFSDDSSE, a peptide that mimics a homophilic binding domain of the neural cell adhesion molecule or NCAM [9] (Fig. 3B). Astrocyte adhesion on surfaces modified with the peptide SEDSDKFISH (a control peptide) was similar to that observed on the reference aminophase glass substrate. These results provide the first evidence that recognition of KHIFSDDSSE by astrocytes is sequence specific (Fig. 3B).

## 4. Discussion

Because integrins are the major molecular mechanism through which cells recognize and respond to extracellular matrix proteins (such as fibronectin, collagen, and laminin), peptides patterned after integrin ligands have received almost exclusive attention in biomaterial design. Alternative molecular mechanisms of cellular interactions with the extracellular environment have only recently been explored in the context of biomaterial surface modifications [12,13]. The present study provides the first evidence that bioactive domains of cell–cell communication proteins (such as NCAM), when immobilized on synthetic materials, can be used to modulate astrocyte adhesion. Since adhesion is the critical prerequisite for subsequent functions of anchorage-dependent cells, adhesion of astrocytes to substrates modified with immobilized KHIFSDDSSE may regulate other cell functions such as proliferation; support of this hypothesis is provided by literature reports that soluble NCAM inhibits astrocyte proliferation both in vivo and in vitro [14,15].

In addition to the importance of the findings of the present study in cellular physiology, these results could lead to improved biomaterials for prostheses in the central nervous system. Specifically, KHIFSDDSSE may be used to modify the surfaces of implant materials to promote astrocyte adhesion (improving anchorage of prosthetic devices into neural tissue), while concurrently inhibiting astrocyte proliferation at the site of implantation (a cellular function associated with the gliotic response). Furthermore, modification of implant material surfaces with immobilized KHIFSDDSSE could also minimize fibroblast adhesion, thus controlling cellular responses that could complicate remodeling of injured nervous tissue. It should be noted, however, that a strategy for designing the definitive proactive material for applications in the nervous system could not be complete without consideration of neuron function. The present study provides some insight in this respect, namely, that adhesion of both astrocytes and fibroblasts was not enhanced on surfaces modified with either IKVAV or YIGSR (Fig. 3), two peptides known to promote neuron attachment, survival and axonal outgrowth on biomaterial surfaces [6,7].

The results of the present study have major implications for the design of the next generation of biomaterials for implant devices with electrically active components. For example, IKVAV and/or YIGSR may be used to selectively promote neuron (but not fibroblast or astrocyte) attachment onto specific regions of material surfaces in order to establish and maintain electrical connections between functional neural prostheses and neurons. Such interactions between implant devices and surrounding brain tissue are essential for proper and long-term function of prostheses in the central nervous system.

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## References

[1] Berry M, Maxwell WL, Logan A, Mathewson A, McConnel P, Ashusrt DE, Thomas GH. Deposition of scar tissue in the central nervous system. Acta Neurochirurgica Suppl (Wein) 1983;32:31–53.

[2] Krikorian JG, Guth L, Donati EJ. Origin of connective tissue scar in the transected rat spinal cord. Exp Neurol 1981;72:698–707.

[3] Turner JN, Shain W, Szarowski DH, Andersen M, Martins S, Isaacson M, Craighead HG. Cerebral astrocyte response to micromachined silicon implants. Exp Neurol 1999;156:33–49.

[4] Massia SP, Hubbell JA. Covalent immobilization of Arg-Gly-Asp- and Tyr-Ile-Gly-Ser-Arg-containing peptide to obtain well-defined cell-adhesive substrates. Anal Biochem 1990;187:292–301.

[5] Olbrich KC, Andersen TT, Blumenstock FA, Bizios R. Surfaces modified with covalently-immobilized adhesive peptides affect fibroblast population motility. Biomaterials 1996;17:759–64.

[6] Saneinejad S, Shoichet MS. Patterned glass surfaces direct cell adhesion and process outgrowth of primary neurons of the central nervous system. J Biomed Mater Res 1998;42:13–9.

[7] Tong YW, Shoichet MS. Peptide surface modification of poly(tetrafluoroethylene-co-hexafluoropropylene) enhances its interaction with central nervous system neurons. J Biomed Mater Res 1998;42:85–95.
[8] Dee K, Rueger DC, Andersen TT, Bizios R. Conditions which promote mineralization at the bone–implant interface: a model in vitro study. Biomaterials 1996;17:209–15.
[9] Rao Y, Wu X, Gariepy J, Rustishauser U, Siu C. Identification of a peptide sequence involved in homophilic binding in the neural cell adhesion molecule NCAM. J Cell Biol 1992;118: 937–49.
[10] Davis-Cox MI, Turner JN, Szarowski D, Shain WG. Phorbol ester-stimulated stellation in primary cultures of astrocytes from different brain regions. Microsc Res Tech 1994;29:319–27.
[11] Kouvroukoglou S, Dee KC, Bizios R, McIntire LV, Zygourakis K. Endothelial cell migration on surfaces modified with immobilized adhesive peptides. Biomaterials 2000;21: 1725–33.
[12] Dee KC, Andersen TT, Bizios R. Design and function of novel osteoblast-adhesive peptides for chemical modification of biomaterials. J Biomed Mater Res 1998;40:371–7.
[13] Rezania A, Healy KE. Biomimetic peptide surfaces that regulate adhesion, spreading, cytoskeletal organization, and mineralization of the matrix deposited by osteoblast-like cells. Biotechnol Process 1999;15:19–32.
[14] Krushel LA, Sporns O, Cunningham BA, Crossin KL, Edelman GM. Neural cell adhesion molecule (N-CAM) inhibits astrocyte proliferation after injury to different regions of the adult rat brain. Proc Nat Acad Sci USA 1995;92:4323–7.
[15] Sporns O, Edelman GM, Crossin KL. The neural cell adhesion molecule (N-CAM) inhibits proliferation in primary cultures of rat astrocytes. Proc Nat Acad Sci USA 1995;92:542–6.