Three-dimensional Migration of Neurites Is Mediated by Adhesion Site Density and Affinity*

Jason C. Schense†§ and Jeffrey A. Hubbell¶‡

From the †Department of Materials and Institute for Biomedical Engineering, Swiss Federal Institute of Technology and the University of Zurich, Zurich CH-8044, Switzerland and the ¶Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125

Three-dimensional neurite outgrowth rates within fibrin matrices that contained variable amounts of RGD peptides were shown to depend on adhesion site density and affinity. Bi-domain peptides with a factor XIIIa substrate in one domain and a RGD sequence in the other domain were covalently incorporated into fibrin gels during coagulation through the action of the transglutaminase factor XIIIa, and the RGD-dependent effect on neurite outgrowth was quantified, employing chick dorsal root ganglia cultured two- and three-dimensionally within the modified fibrin. Two separate bi-domain peptides were synthesized, one with a lower binding affinity linear RGD domain and another with a higher binding affinity cyclic RGD domain. Both peptides were cross-linked into fibrin gels at concentrations up to 8.2 mol of peptide/mol of fibrinogen, and their effect on neurite outgrowth was measured. Both two- and three-dimensional neurite outgrowth demonstrated a bi-phasic dependence on RGD concentration for both the linear and cyclic peptide, with intermediate adhesion site densities yielding maximal neurite extension and higher densities inhibiting outgrowth. The adhesion site density that yielded maximal outgrowth depended strongly on adhesion site affinity in both two and three dimensions, with lower densities of the higher affinity ligand being required (0.8–1.7 mol/mol for the linear peptide versus 0.2 mol/mol for the cyclic peptide yielding maximum neurite outgrowth rates in three-dimensional cultures).

Cell adhesion and migration in two and three dimensions play important roles in a host of physiological phenomena, including neurite extension (1), angiogenesis (2), and immune responses (3). The adhesion of cells within their environment is controlled primarily by the binding of cell surface receptors to short, exposed protein domains in the extracellular matrix. An important receptor family involved in adhesion and migration is the integrins, a class of heterodimeric receptors that interact with both extracellular matrix and cell-surface ligands (4, 5). Each unique dimeric receptor binds to a particular subset of ligands, which can often be mimicked with immobilized short peptides (6). One such short peptide that binds to integrins and influences subsequent cell adhesion and migration is RGD, a motif present in many proteins, including fibronectin, laminin, collagen, and fibrinogen (7). This sequence has been shown to be recognized by α5β1, αvβ3 (8), and several other integrin heterodimer pairs.

Adhesion and migration upon a surface are distinct processes that depend on related molecular mechanisms. Cell adhesion involves the dynamic interaction between cell surface receptors and adhesion ligands on the supporting matrix (9, 10), with the strength of this attachment being related to the concentration of cell-surface receptors, the concentration of ligands available, and the specific binding affinity of the receptor ligands and pairs (11). Migration of cells upon a material is an analogous phenomenon, with the rate of cell migration depending on both ligand density and binding affinity as well as the density of corresponding cell surface receptors. As cells move within their environment, they must constantly detach at the trailing edge and reattach at the leading edge to nearby ligands (12, 13). The corresponding rate of cell migration upon a surface depends on the simultaneous detachment and attachment to nearby ligands, causing extreme ligand densities (both high and low) to inhibit one of these two processes and subsequently limit cell migration rates. The outgrowth of neurites is analogous to cell migration (14), with receptor cluster morphology (15) and receptor dynamics at the growth cone (16) controlling the process.

Cell migration and neurite extension within a three-dimensional matrix involves a more complex balance of cellular activity than migration upon a two-dimensional surface, with cell-associated proteolysis playing a key role in three-dimensional migration. When cells are present inside a fibrin matrix for example, the porosity of the fibrous network is significantly smaller than the average cell and even neurite size. Therefore, in order for cells or neurites to migrate within fibrin, it is necessary that the surrounding matrix be remodeled, and this is accomplished through the action of the protease plasmin (17–19). While adhesion signals still play an important role in three-dimensional migration, the dependence also on proteolysis complicates a one-to-one correlation between two- and three-dimensional cell migration.

The focus of this work was neurite migration within a fibrin matrix with controlled adhesive characteristics. Through the action of the transglutaminase, factor XIIIa, bi-domain peptides were incorporated within a three-dimensional fibrin matrix at precise and predetermined concentrations (20). A bi-phasic response was observed in both two- and three-dimensional neurite outgrowth, with intermediate RGD adhesion site densities leading to fastest outgrowth. Outgrowth rates depended strongly on binding affinity with a lower number of higher affinity binding sites leading to a similar response as a higher number of lower affinity ligands.

* This work was funded by Grant 31-52261 NFP 38 from the Swiss National Science Foundation and a gift from F. Hoffmann-La Roche AG and Novartis Pharma AG. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Institute for Bioengineering, ETH-Zurich and University of Zurich, Moussonstrasse 18, CH-8044 Zurich, Switzerland. Tel.: 41-1-632-45-75; Fax: 41-1-632-12-14; E-mail: hubbell@biomed.mat.ethz.ch.

© 2000 by The American Society for Biochemistry and Molecular Biology, Inc.

To whom correspondence should be addressed: Institute for Biomedical Engineering, ETH-Zurich and University of Zurich, Moussonstrasse 18, CH-8044 Zurich, Switzerland. Tel.: 41-1-632-45-75; Fax: 41-1-632-12-14; E-mail: hubbell@biomed.mat.ethz.ch.

Printed in U.S.A.
Three-dimensional Migration of Neurites

EXPERIMENTAL PROCEDURES

Peptides and Proteins—dLNQEQVSPLRGD-NH₂, and dLNQEQVSPLRGD-NH₂ (d = dansyl; for clarity, the factor XIIa substrate domain (21) is shown in italics) were synthesized on solid resin using an automated peptide synthesizer (9050 Pep Plus Synthesizer, Millipore, Framingham, MA) with standard 9-fluorenemethylxycarbonyl chemistry (22). These peptides were labeled with a fluorescent probe by including dansylated leucine (Sigma, Buchs, Switzerland) at the amino terminus of the peptide. Hydrophobic scavengers and cleaved protecting groups were removed by precipitation of the peptide in cold diethyl ether and dissolution in deionized water. After lyophilization, these two peptides were redisolved in 0.03 M Tris-buffered saline (TBS, pH 7.0) and analyzed using high performance liquid chromatography (Waters, Milford, CT) on a size exclusion column with TBS, pH 7.0, as the running buffer. The cyclic peptide, NH₂-LNQEQVSPFRGDNRC- [CRGDNRC] (cyclic ring shown in brackets), was purchased from Genemed (South San Francisco, CA) at greater than 85% purity.

Fibrinogen solutions were prepared by dissolving fibrinogen (Fluka, Buchs, Switzerland) in deionized water at 8 mg/ml for 4 h. Dialysis (Spectrum, 6000–8000 MWCO; Laguna Hills, CA) versus 400 volumes of TBS, pH 7.4, for 24 h was used to exchange salts present in the protein solution. The resulting solution was then sterilized by filtering through a 0.22-μm syringe filter. The final fibrinogen concentration was determined by measuring the absorbance of the protein solution at 280 nm. TBS solutions at pH 7.0 that were made with either 50 mM Ca²⁺ or without any Ca²⁺ were sterilized by filtration through a 0.22-μm filter. These solutions were made by dissolving fibrinogen (Sigma, Buchs, Switzerland) in TBS at pH 7.0 at a concentration of 1 mg/ml.

Fibrin formation—Fibrin gels were made in flat-bottomed 24-well culture plates by mixing the four components such that final concentrations obtained were 1) 3.6 mg/ml fibrinogen, 2) 2.5 mM Ca²⁺, 3) 2 μg/ml of thrombin, and 4) various amounts of added peptide. Fibrin gels without added exogenous peptides (all values were normalized to this level of neurite extension within native fibrin or with cross-linked dLNQEQVSPLRGD-NH₂, dLNQEQVSPLRGD-NH₂, or LNQEQVSPFRGDNRC) were polymerized with a DRG within the gel in the center of a 35-mm Petri dish. The DRGs were cultured in 2 ml of neuronal medium, which was replaced once at 6 h to remove free peptide. Samples were stained at 24 h, prior to imaging, by adding 2 μl of a 5 mg/ml stock solution of fluorescein diacetate in acetone to 1 ml of TBS. Approximately 50 images were taken at 10-μm intervals, and a composite image was assembled using Imaris (Bitplane, Zurich, Switzerland) image processing software on an Indigo2 extreme Silicon Graphics Workstation (Silicon Graphics, Mountain View, CA).

Statistics—The outgrowth for each individual ganglion was normalized by the mean neurite outgrowth observed for ganglia simultaneously cultured in or on unmodified fibrin. Experiments were completed in triplicate and these normalized values were then averaged to determine the change in outgrowth in relation to outgrowth observed with unmodified fibrin. Statistical analyses of these data were performed using Statview 4.5 (Abacus, Berkeley, CA). Comparitive analyses were completed using analysis of variance with Fisher’s protective t test at a 95% confidence level. Mean values and standard errors of the mean are shown.

Linear curve fits were calculated for the data on inhibition with soluble peptides using Cricket Graph III (Computer Associates), which employs a linear least squares regression method. The analysis was limited to the linear domain of the inhibition curves and was used to determine the relative binding affinities of the two RGD peptides employed.

RESULTS

Competitive Inhibition of Three-dimensional Neurite Outgrowth—As a measure of the relative binding affinity of the two RGD peptides employed, the effect of each peptide as a soluble competitive inhibitor of the three-dimensional neurite outgrowth from day 8 chick DRGs within fibrin was studied. We tested the linear peptide, dLNQEQVSPLRGD, was added to the culture medium, after fibrin polymerization, as a competitive inhibitor in a concentration series. Neurite outgrowth decreased in a dose-dependent manner (Fig. 1). At the highest concentration tested, 1.05 mM, the peptide decreased neurite outgrowth by 25 ± 7% at 24 h relative to the outgrowth within unmodified
RGD peptide. The control sequence, RDG, proved to have no effect on neurite outgrowth within three-dimensional fibrin matrices. Peptide was added in the culture medium as a soluble competitive inhibitor for endogenous RGD in the fibrin matrix. Neurite outgrowth from day 8 DRGs was measured and normalized to outgrowth in unmodified fibrin at 24-h culture time (nonnormalized value for unmodified fibrin is 0.207 ± 0.010 mm). Experiments were performed in triplicate with six DRGs per replicate, and mean values and S.E. are shown; * denotes p < 0.05 compared with unmodified fibrin. At each concentration point, the cyclic peptide statistically inhibited neurite outgrowth to a greater extent than did the linear peptide, demonstrating that the cyclic RGD is a higher affinity ligand than the linear RGD. The incorporation of the inactive control, RDG, did not affect neurite outgrowth at any of the concentrations tested. Curve fits were calculated for the two RGD peptides and are shown in dashed lines over the concentrations points used in this calculation. The ratio of the binding affinities for the cyclic RGD to the linear RGD (the ratio of the slope of the two linear curve fits) is 9.0. Fibrin in the absence of soluble peptide inhibitor (p < 0.05). The cyclic peptide LNQEQVSPD(CRGDNRC) decreased neurite outgrowth to a greater extent compared with the linear peptide, e.g. by 46 ± 6% at 24 h when 0.40 mM peptide was added to the medium. Furthermore, at every concentration point, the cyclic RGD peptide inhibited the growth of neurites to a greater extent than did the linear RGD peptide (p < 0.05). Linear curve fits were calculated for the two active RGD peptides, and it was found that the slope for the curve fit for the cyclic RGD peptide was 9.0 times steeper than that for the curve fit for the linear RGD peptide. The control sequence, RDG, proved to have no effect on three-dimensional neurite outgrowth over the entire concentration range tested (p > 0.52).

Neurite Extension in Two Dimensions with Cross-linked RGD Sites—Fibrin gels were modified by cross-linking various concentrations of linear or cyclic RGD or linear RDG peptide into the gel, and the resulting effect on three-dimensional neurite outgrowth was measured at 24 and 48 h. A bi-phasic response was observed, with the cross-linking of 1.7 mol of linear RGD peptide/mol of fibrinogen enhancing the level of neurite outgrowth by 23 ± 7% at 24 h and 14 ± 5% at 48 h (p < 0.05) (Fig. 3). The incorporation of 4.4 or 8.2 mol of linear RGD peptide/mole fibrinogen decreased the level of neurite outgrowth, such that it was lower than the outgrowth observed within both unmodified fibrin as well as the fibrin modified with lower concentrations of cross-linked peptide (p < 0.05). At the highest incorporated adhesion density of 8.2 mol/mol, the level of neurite outgrowth was reduced by 21 ± 5% at 24 and 18 ± 4% at 48 h relative to outgrowth within unmodified fibrin.

The incorporation of the higher affinity-binding cyclic peptide, LNQEQVSPD(CRGDNRC), into fibrin gels had a similar bi-phasic effect on the outgrowth of neurites, with an overall effect that was shifted to lower peptide densities relative to the effect for the lower affinity linear RGD peptide. Incorporation of 0.2 mol/mol of cyclic peptide enhanced the outgrowth of neurites by 17 ± 7% at 24 and 14 ± 3% at 48 h compared with outgrowth within unmodified fibrin (p < 0.05). Each of the higher cross-linking concentrations tested resulted in a decrease in neurite outgrowth, such that at 0.8 mol/mol, outgrowth was reduced by 25 ± 7% at 24 and 16 ± 4% at 48 h, and at 8.2 mol/mol, neurite outgrowth was reduced by 51 ± 5% at 24 and 25 ± 5% at 48 h relative to outgrowth within unmodified fibrin (p < 0.05). Incorporation of the RDG peptide had no
Three-dimensional Migration of Neurites

The effect of incorporated cyclic (D) or linear (C) RGD peptides and a linear (B) RGD peptide at various concentrations on neurite outgrowth within three-dimensional fibrin matrices. Neurite outgrowth from day 8 DRGs was measured and normalized to outgrowth in unmodified fibrin at 24 (A) and 48 h (B) culture time (nonnormalized value for unmodified fibrin is 0.207 ± 0.010 mm at 24 h and 0.746 ± 0.027 mm at 48 h). Experiments were performed in triplicate with six DRGs per replicate, and mean values and S.E. are shown; * denotes p < 0.05 compared with unmodified fibrin, and ‡ denotes points that are statistically identical. The correlation between neurite outgrowth and cross-linked linear RGD peptide showed a similar bi-phasic effect as seen in two-dimensional RGD-dependent cellular migration. The incorporated cyclic RGD peptide showed a similar effect, with the curve shifted consistently to lower concentrations due to its higher binding affinity. The relative binding affinity ratio calculated from the curve fits of the competitive soluble inhibition experiment (Fig. 1) was used to normalize the linear RGD data at both 24 and 48 h (A). When the linear RGD data are normalized in this manner, both the 24- and 48-h neurite outgrowth are very similar to that observed with the cyclic RGD peptide. The incorporation of the inactive peptide, RDG, did not affect neurite outgrowth at any of the concentrations.

The relative binding affinities of the cyclic and the linear peptides were estimated from the results on competitive inhibition in three-dimensional outgrowth experiments. The cyclic peptide was estimated to bind 9.0 times more strongly. This ratio was used to compare the results in outgrowth with immobilized linear and cyclic peptides in terms of affinity, rather than molar amounts of peptide, by dividing the abscissa for the linear peptide by 9.0. The adjusted results are shown in Fig. 3. The curves, when adjusted according to relative affinity are highly coincident.

The difference in outgrowth induced from the ganglia in the various samples was pronounced enough to be clearly observed. As a demonstration, a representative sample from materials that induced the greatest and the smallest neurite outgrowth are shown in Fig. 4. Samples are shown for the linear peptide where maximal outgrowth (C) and minimal outgrowth (E) were achieved. An identical set are present for the cyclic peptide (D and F, respectively) in addition to representative samples for the two controls, unmodified fibrin (A) and RDG modified fibrin (B).

DISCUSSION

The migration of cells within a three-dimensional matrix is an important component of many cellular processes both in vivo and in vitro. However, the lack of three-dimensional model materials in which the biochemical characteristics can be readily and reproducibly controlled prevents complete understanding of the relevant factors involved. Through the factor XIIIa-mediated modification of fibrin matrices, we have developed a method by which the bioactive character of a degradable matrix can be reproducibly manipulated (20). Using these matrices, the influence that exogenous RGD sequences have on the outgrowth of neurites within a three-dimensional environment was studied, with the effect on neurite outgrowth shown to exhibit a bi-phasic dependence on RGD concentration and binding affinity.

The relative binding affinities of the peptides employed were characterized by competitive inhibition experiments in three-dimensional culture. RGD is found endogenously in fibrin, at an active concentration of 2 mol/mol of fibrinogen (25). The addition of either of the RGD peptides as a soluble inhibitor blocked the activity of these endogenous sequences, resulting in a decrease in neurite outgrowth within the fibrin matrix. A high concentration of the linear RGD peptide (1.05 mM) was required to decrease the outgrowth of neurites. The addition of the cyclic RGD peptide as a soluble competitive inhibitor proved to be much more potent in slowing neurite outgrowth, such that at each tested concentration, the cyclic peptide inhibited neurite outgrowth to a greater extent than did the linear peptide. When the data were correlated to a linear curve fit, it was shown that the curve fit for the cyclic peptide was 9.0 times steeper than that for the linear RGD, indicating that the binding affinity in this system for the cyclic peptide is 9.0 times greater than that for the linear RGD peptide. This greater binding affinity for cyclic RGD peptides over linear RGD peptides has been demonstrated previously (26), with the enhanced binding affinity due to the forced secondary structure occurring from the ring (27). It has been shown that cyclic RGD peptides adopt a conformation similar to the turn between strands in a β sheet, forcing cyclic peptides to mimic a common structural motif for native RGD sites (28).

Two-dimensional neurite outgrowth demonstrated a bi-phasic dependence on RGD site density, consistent with the outgrowth of neurites being a form of cell migration. Much experimental and modeling work has been completed showing that the effect of adhesion site surface concentration on the motility of cells, such as smooth muscle cells, displays a bi-phasic response (12, 29). The extent of the bi-phasic response in two dimensions depends on the adhesion site and receptor surface concentrations and the degree to which cell surface receptors are polarized toward the leading edge (11). Evidence suggests that the migration of the neuronal growth cone, and therefore the overall neurite outgrowth rate, represents a similar phenomenon (16). By incorporating RGD into a fibrin matrix, it has been demonstrated that the neurite outgrowth from chick DRGs displays such a bi-phasic dependence on RGD density, with a moderate peptide density eliciting the highest outgrowth rate. These results are thus consistent with the migration of neuronal growth cones, and therefore neurite outgrowth, being directly analogous to the migration of individual cells.

RGD peptides cross-linked into fibrin gels demonstrated a bi-phasic adhesive-like character in three-dimensions. The
striking similarity between the results in three-dimensional and two-dimensional outgrowth experiments suggest that the rate-controlling phenomena are similar between the two cases. This is very interesting, because in two-dimensional cultures proteolysis plays a minor role, whereas in the three-dimensional cultures proteolysis is required for neurite outgrowth (17). The similarity in results suggests that proteolysis of the fibrin matrix is not the rate-controlling phenomenon, but rather that adhesion and migration biomechanics control the overall outgrowth rate. The results also suggest, although indirectly, that the density of exogenous RGD sites did not influence neurite outgrowth rates primarily by modulating local protease activity. If they had done so, then the site density-dependent bi-phasic response that was observed in three-dimensional outgrowth (where proteolysis is requisite) would not have been observed in two-dimensional outgrowth (where proteolysis is not requisite) (17).

A strong dependence of both two-dimensional and three-dimensional neurite outgrowth upon adhesion site binding affinity was observed. The phenomena observed for the two RGD peptides employed were similar in both form and extent, but with the response curve being markedly shifted to lower concentrations for the higher affinity cyclic RGD peptide such that a significantly lower incorporated density of cyclic RGD was required to induce maximal neurite outgrowth. To better quantify this phenomenon, the three-dimensional outgrowth was presented for the two RGD peptides in terms of comparable adhesive density rather than peptide density. This was accomplished by first calculating the relative binding affinities for the two RGD peptides from the competitive inhibition study. This ratio was calculated to be 9.0 and was then used to correlate the effects of the two RGD sequences by dividing the incorporated density for the linear peptide by this relative binding affinity ratio. When this was done, the RGD-dependent neurite outgrowth for the adjusted linear RGD peptide (Fig. 3, △) was strikingly similar to that for the cyclic RGD peptide (Fig. 3, △). Therefore, it is very likely that the lower density of the higher affinity cyclic peptide, which induced maximal neurite outgrowth represents a total adhesive character similar to the higher density of the lower affinity linear peptide, which induced maximal neurite outgrowth as well. This is further supported in that the levels of enhancement of neurite outgrowth for these two formulations are of similar intensities. Within the concentration series tested, both RGD peptides demonstrated a similar adhesive-like character in three-dimensional migration, and this character corresponds directly to effects seen in the two-dimensional studies.

It has been recently demonstrated that integrin number in the growth cone is regulated by receptor occupancy. Neurons were purified from embryonic chick DRGs and plated on surfaces coated with either a low or high concentration of the extracellular matrix protein laminin or the extracellular matrix protein fibronectin. When the amount of a laminin-specific integrin receptor, α5β1, was analyzed, it was shown to have increased in the presence of low concentrations of laminin and decreased in the presence of high concentrations of laminin relative to similar neurons plated on fibronectin coated surfaces (30). This compensatory mechanism is hypothesized to enable the neurite to migrate over a very wide range of adhesion site densities. This compensatory mechanism may be less at play in the fibrin model employed, as a clear response of outgrowth rate to the adhesive characteristics of the matrix was observed. It also may be possible that this compensatory effect is even less at play in three-dimensional outgrowth, since high immobilized concentrations of the high affinity cyclic RGD ligand inhibited neurite outgrowth much more extensively in three-dimensional culture than in two-dimensional culture, approximately 2-fold more.

The addition of exogenous adhesion sites within a fibrin matrix is sufficient for enhancing two- and three-dimensional neurite outgrowth. The overall process of neurite outgrowth involves the simultaneous coordination of many individual processes, including binding to both adhesion and other bioactive sites, polymerization of actin, formation of new membrane, and

---

**Fig. 4. Images of DRGs cultured within unmodified fibrin (A), fibrin with 8 mol of RGD/mol of fibrinogen (B), fibrin with 1.7 mol of linear RGD/mol of fibrinogen (C), fibrin with 0.2 mol of cyclic RGD/mol of fibrinogen (D), fibrin with 8 mol of linear RGD/mol of fibrinogen (E), and fibrin with 8 mol of cyclic RGD/mol of fibrinogen (F).** Confocal scanning laser microscopy of DRGs was performed using 10x magnification with approximately 50 images taken at 10-μm intervals shown as an extended focus projection. The scale bar represents 100 μm. The ganglia grown within unmodified fibrin show a similar level of outgrowth to that seen within RDG-modified fibrin gels. This level is less than that observed in ganglia grown within fibrin with low levels of incorporated RGD peptides (C and D) but greater than that observed in ganglia grown within fibrin with high levels of incorporated RGD peptides (E and F).
intracellular migration of proteins within the axon (31–33). While each of these components are necessary for neurite migration, it is possible that modulating a single factor might not be sufficient to increase the overall rate of neurite migration. By cross-linking various concentrations of exogenous RGD peptides within a fibrin matrix formed from physiological fibrinogen concentrations, materials have been developed whereby the likely change is in the adhesive character of the material. These materials were able to modestly increase the level of neurite outgrowth, demonstrating that adhesion of the migrating neurite growth cone is a significant and rate controlling factor, even if it constitutes only one part of a complicated process.

The use of biomaterials for tissue regeneration, such as the promotion of nerve healing, is a promising field of research. By developing materials with specific adhesive characteristics, it is possible to control and enhance the level of cell migration within the material. This may lead to more effective forms of therapy in many areas of tissue engineering, such as promoting better integration at the tissue-biomaterial interface, promoting cell infiltration, and controlling morphogenesis in tissue regeneration.

Acknowledgments—We thank Dr. M. Höchli and Prof. T. Bach of the Electronmicroscopical Laboratory at the University of Zurich for their assistance with confocal scanning laser microscopy.

REFERENCES
1. Jessell, T. M. (1991) in Principles of Neural Science (Kandel, E. R., Schwartz, J. H., and Jessell, T. M., eds) pp. 908–928, Simon and Schuster, East Norwalk, CT
2. Beck, L., and D’Amore, P. A. (1997) FASEB J. 11, 365–373
3. Benelli, R., Mortarini, R., Anichini, A., Giusemiaglio, D., Noonan, D. M., Montalti, S., Tacchetti, C., and Albinii, A. (1998) AIDS 12, 261–268
4. Hynes, R. O. (1992) Cell 69, 11–25
5. Yamada, K. M. (1997) Matrix Biol 16, 137–141
6. Massia, S. P., and Hubbell, J. A. (1991) J. Cell Biol. 114, 1089–1100
7. Yamada, K. M. (1991) J. Biol. Chem. 266, 12809–12812
8. Pierschbacher, M. D., and Ruoslahti, E. (1984) Nature 309, 30–33
9. Ruoslahti, E. (1991) J. Clin. Invest. 87, 1–5
10. Lander, A. (1989) Trends Neurosci. 12, 189–195
11. Palecek, S. P., Loftus, J. C., Ginsberg, M. H., Lauffenburger, D. A., and Horwitz, A. F. (1997) Nature 385, 537–540
12. DiMilla, P. A., Stone, J. A., Quinn, J. A., Alhelda, S. M., and Lauffenburger, D. A. (1993) J. Cell Biol. 122, 729–737
13. Lauffenburger, D. A., and Horwitz, A. F. (1996) Cell 84, 359–369
14. Bixby, J. L., and Harris, W. A. (1991) Annu. Rev. Cell. Biol. 7, 117–159
15. Gomez, T. M., Roche, F. K., and Letourneau, P. C. (1996) J. Neurosci. 29, 18–34
16. Schmidt, C. E., Dai, J., Lauffenburger, D. A., Sheetz, M. P., and Horwitz, A. F. (1995) J. Neurosci. 15, 3490–3497
17. Herbert, C. B., Bittner, G. D., and Hubbell, J. A. (1996) J. Comp. Neurol. 365, 380–391
18. Shea, T. B., and Beermann, M. L. (1992) FEBS Lett. 307, 190–194
19. Pittman, R. N., and Ruoslahti, E. (1990) Proc. Natl. Acad. Sci. USA 87, 8287–8291
20. Schense, J. C., and Hubbell, J. A. (1999) Bioconjug. Chem. 10, 55–61
21. Ichinose, A., Tamaki, T., and Aoki, N. (1982) FEBS Lett. 153, 369–371
22. Fields, G., and Noble, R. (1989) J. Cell Biol. 109, 231–241
23. Dellenback, R. J., and Chien, S. (1992) Proc. Natl. Acad. Sci. USA 89, 2473–2477
24. Varon, S. J., Nomura, J., Perez-Polo, J. R., and Sheetz, M. P. (1992) J. Cell Biol. 117, 1–10
25. Koivunen, E., Wang, B., and Ruoslahti, E. (1996) Biochem. Biophys. Res. Commun. 223, 977–982
26. Varon, S. J., Nomura, J., Perez-Polo, J. R., and Sheetz, M. P. (1992) J. Cell Biol. 117, 1–10
27. Jones, C. M., and Letourneau, P. C. (1997) J. Neurosci. 17, 555–565
28. Condic, M. L., and Letourneau, P. C. (1997) J. Neurosci. 17, 555–565
29. Bixby, J. L., and Harris, W. A. (1991) Annu. Rev. Cell. Biol. 7, 117–159
30. Condic, M. L., and Letourneau, P. C. (1997) J. Neurosci. 17, 555–565
31. Luckenbill-Edds, L. (1997) Brain Res. Brain Res. Rev. 23, 1–27
32. Fu, S. Y., and Gordon, T. (1997) Mol. Neurobiol. 14, 67–116
33. Bixby, M. A., and Tetzlaff, W. (1992) Mol. Neurobiol. 6, 107–123