Cell adhesion molecule DM-GRASP presented as nanopatterns to neurons regulates attachment and neurite growth†

Karsten Thelen,‡[a] Tobias Wolfram,‡[bc] Bettina Maier,[a] Steffen Jährling,[a] Ahmed Tinazli,[d] Jacob Piehler,[d] Joachim P. Spatz[b] and G. Elisabeth Pollerberg*[a]

Received 14th May 2007, Accepted 28th September 2007
First published as an Advance Article on the web 12th October 2007
DOI: 10.1039/b707250c

Adhesion and neurite formation of neurons and neuroblastoma cells critically depends on the lateral spacing of the cell adhesion molecule DM-GRASP offered as nanostructured substrate.

Cell adhesion molecules of the immunoglobulin superfamily (IgSF-CAMs) are crucial for a variety of processes during the formation of the nervous system such as cell adhesion, migration, differentiation, neurite formation, and regeneration. IgSF-CAMs are integral plasma membrane proteins which interact with IgSF-CAMs of the same type in opposing cell surfaces (homophilic *trans*-interaction) thereby serving as both receptor and ligand. IgSF-CAMs are moreover capable of interactions with other IgSF-CAMs (heterophilic interactions) and also interact in the plane of the plasma membrane (*cis*-interactions). Due to technical limitations it is yet unknown how the spatial arrangement of IgSF-CAMs affects their impact on cellular functions. Recently, a substrate patterning technique based on self-assembling di-block copolymer micelles was developed allowing for the assembly of extended areas of highly regular arranged gold nanodot patterns.[1,2] Coupling the entire extracellular domain (ed) of an IgSF-CAM to the nanodots and employing these nanopatterns as cell culture substrates, we aimed at the manipulation of the IgSF-CAM spacing in the plasma membrane by homophilic *trans*-interactions. The IgSF-CAM DM-GRASP (BEN, SC1) contains five extracellular Ig-like domains, a transmembrane region, and a short cytoplasmic domain.[3,4] DM-GRASP is capable of homophilic and heterophilic (with IgSF-CAM L1) interactions.[4,5] During development of the nervous system, DM-GRASP is present on neurons' extending processes (neurites) and has been found to be involved in their navigation and fasciculation.[6,7] On conventional DM-GRASP substrates, *i.e.* DM-GRASP-coated glass, neurite formation and elongation of dorsal root ganglion (DRG) neurons has been reported.[5] In these experiments, however, neither concentration, spacing, and orientation of DM-GRASP nor the deposition of other proteins shed by the cells was controlled.

To functionalize hexagonal nanodot patterns interspaced 58 nm and 73 nm (Fig. 1A, B), the histidine-tag at the carboxyl-terminal end of edDM-GRASP was bound to Ni^{2+}-NTA coupled to the gold nanodots thus exhibiting the same orientation at the N-terminus as DM-GRASP in the cell membrane (Fig. 1C). Histidine-tagged edDM-GRASP, released by HEK293 cells, was purified by metal-affinity chromatography. Analysis of the isolated proteins by SDS-PAGE revealed a single, broad band at about 100 kDa, indicating the purity and normal degree of glycosylation of the secreted edDM-GRASP (Fig. 1D); Western blot analysis

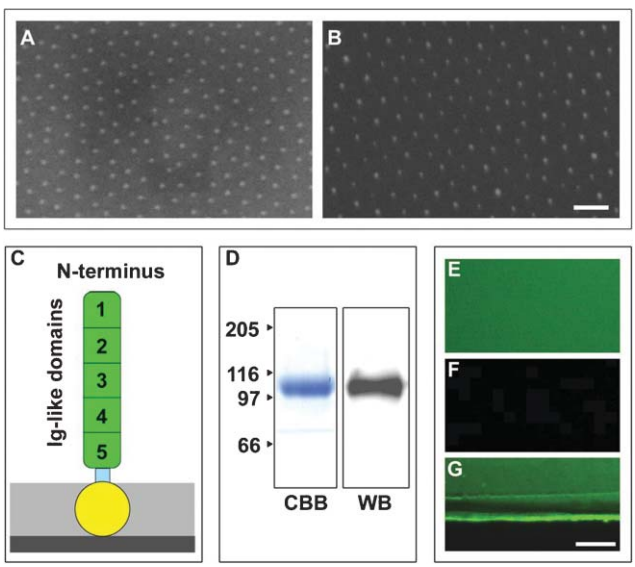

Fig. 1 Gold nanodots biofunctionalized with DM-GRASP. Scanning electron microscopy images show 6 nm large gold nanodots on glass coverslips with interdot distances of 58 nm (A) and 73 nm (B). The extracellular domain of DM-GRASP consisting of five Ig-like domains (green) is coupled *via* a histidine-tag and NTA-thiol linker (blue) to gold nanodots (yellow), the interspace between the nanodots is covered with PEG (grey) (C). (D) Coomassie Brilliant Blue-stained SDS-PAGE (CBB) and Western blot (WB) of histidine-tagged DM-GRASP expressed by HEK293 cells show the purity of the recombinant protein with an apparent molecular weight of about 100 kDa. Immunofluorescence staining using a polyclonal DM-GRASP antibody demonstrates that DM-GRASP is confined to the area covered by nanodots and exhibits a homogenous distribution (E). No DM-GRASP is detected in the non-nanodot area (F). Only in the immediate vicinity of the border of the nanodot and non-nanodot areas, is DM-GRASP found at higher and lower levels (G). Scale bars: 100 nm in (A, B), and 10 μm in (E–G).

[a]*University of Heidelberg, Department of Developmental Neurobiology, Institute of Zoology, Im Neuenheimer Feld 232, 69120 Heidelberg, Germany*
[b]*Max-Planck-Institute for Metals Research, Dept. New Materials and Biosystems, & University of Heidelberg, Dept. of Biophysical Chemistry, Heisenbergstrasse 3, 70569 Stuttgart, Germany*
[c]*Institute for Molecular Biophysics, The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609, USA*
[d]*Institute of Biochemistry, Biocenter N210, Max-von-Laue-Straße 9, 60438 Frankfurt, Germany*
† Electronic supplementary information (ESI) available: Experimental procedures; figure showing restriction of adhesion of DRG cells and neurite growth of DRG neurons to nanostructured area functionalized with edDM-GRASP. See DOI: 10.1039/b707250c
‡ These authors contributed equally to this work.

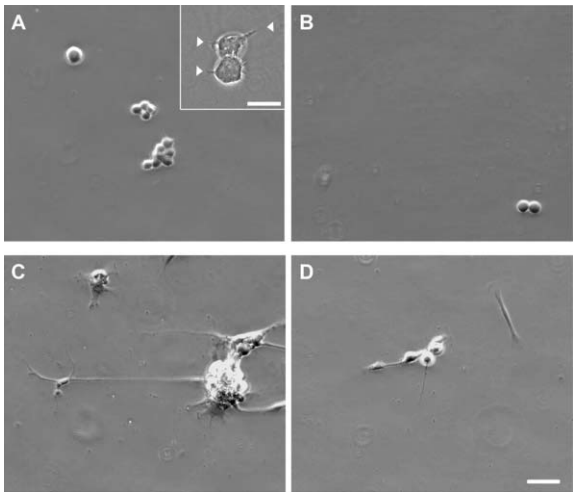

Fig. 2 Cell adhesion on DM-GRASP nanopatterns. N2a cells bind to DM-GRASP offered as 58 nm patterns (A); on 73 nm patterns, adhesion of N2a cells is less efficient (B). Insert in (A) shows N2A cells with protrusions in a higher magnification. DRG cells bind to both 58 nm (C) and 73 nm (D); on the narrow-spaced substrate larger aggregates are formed. Flat mesenchymal cells are visible at the margin of the aggregates. On the narrow-spaced patterns, long neurites are present whereas on the wide-spaced patterns, protrusions are rare and short. Scale bar: 50 μm in (D) and 20 μm in insert (A).

using a DM-GRASP-specific antibody clearly shows that the 100 kDa band represents secreted edDM-GRASP.

Immunofluorescence labeling shows that edDM-GRASP is restricted to the area covered with nanodots (structured area) and is absent from the area containing only polyethylene glycol (PEG, non-structured area) (Fig. 1E, F). This demonstrates that the passivation by PEG is highly efficient and prevents unspecific deposition of DM-GRASP. The DM-GRASP labeling moreover reveals its homogeneous distribution over the entire structured area, with an enrichment only at the border of the unstructured area (Fig. 1G). The nanopatterns thus represent a cell culture substrate which displays edDM-GRASP in a highly defined spacing, controlled amino-carboxyl-terminal orientation, and without any unspecific depositions.

To investigate the impact of the lateral spacing of substrate-DM-GRASP on neuronal cells, neuroblastoma cells expressing DM-GRASP (DM-GRASP-N2a cells) were cultured on 58 and 73 nm substrate patterns for 30 hours. DM-GRASP-N2a cells are able to attach to both nanopatterns, however with distinct efficiencies (representative images are presented in Fig. 2A, B): On the narrow-spaced edDM-GRASP pattern, about ten times more cells adhere compared to the wide-spaced pattern (316 and 30 cells/10 mm^2, respectively). On both surfaces, DM-GRASP-N2a cells form only short protrusions (up to 20 μm in length) indicating that edDM-GRASP cannot promote substantial neurite extension. DM-GRASP-N2a cells are capable of sending out long neurites, *e.g.* on conventional Laminin substrates where 22% of the cells form neurites longer than 20 μm which, however, might be due to shed proteins. The density of edDM-GRASP-functionalized dots employed in these experiments thus positively correlates with adhesion but not with neurite formation of DM-GRASP-N2a cells.

In order to analyze the effect of spacing of DM-GRASP offered as a substrate on primary neurons endogenously expressing DM-GRASP, DRG single cell cultures containing both sensory neurons and mesenchymal cells were analyzed (Fig. 2C, D; Table 1). Histidine-edDM-GRASP was offered as 58 and 73 nm nanopatterns and cells were kept in culture for 30 hours. No cells or neurites were found in the non-structured area (Supplemental Fig. 1†). Almost five times more cells adhere to the 58 nm pattern compared to the 73 nm one. On 58 nm edDM-GRASP nanopatterns the percentage of neurons compared to non-neuronal cells is 2.5-fold higher than on 58 nm nanopatterns functionalized with

Table 1 Quantification of cell attachment. The number of cells and cell types were determined by marker expression, nuclear staining, and morphological criteria; the number of clusters is given in brackets. Single cells are without any contact to other cells, small clusters consist of 2–5 cells, medium-sized clusters of 6–30 cells, and large clusters of more than 30 cells

Neurons										
	Single cells	In small clusters		In medium clusters		In large clusters		Total		% of total	% in cluster
58 nm	12	27	(23)	137	(22)	270	(6)	446	(51)	77	97
73 nm	5	14	(10)	65	(4)	0	(0)	84	(14)	72	94
PLL	90	9	(9)	0	(0)	0	(0)	99	(9)	54	9
PLL/Lam	67	22	(17)	0	(0)	0	(0)	89	(17)	66	25
Mesenchymal cells										
	Single cells	In small clusters		In medium clusters		In large clusters		Total		% of total	% in cluster
58 nm	32	39	(23)	42	(22)	19	(6)	132	(51)	23	76
73 nm	13	11	(10)	9	(4)	0	(0)	33	(14)	28	61
PLL	76	10	(9)	0	(0)	0	(0)	86	(9)	46	12
PLL/Lam	34	12	(17)	0	(0)	0	(0)	46	(17)	34	26
All cells										
	Single cells	In small clusters		In medium clusters		In large clusters		Total		% of total	% in cluster
58 nm	44	66	(23)	179	(22)	289	(6)	578	(51)		92
73 nm	18	25	(10)	74	(4)	0	(0)	117	(14)		85
PLL	166	19	(9)	0	(0)	0	(0)	185	(9)		10
PLL/Lam	101	34	(0)	0	(0)	0	(0)	135	(0)		25

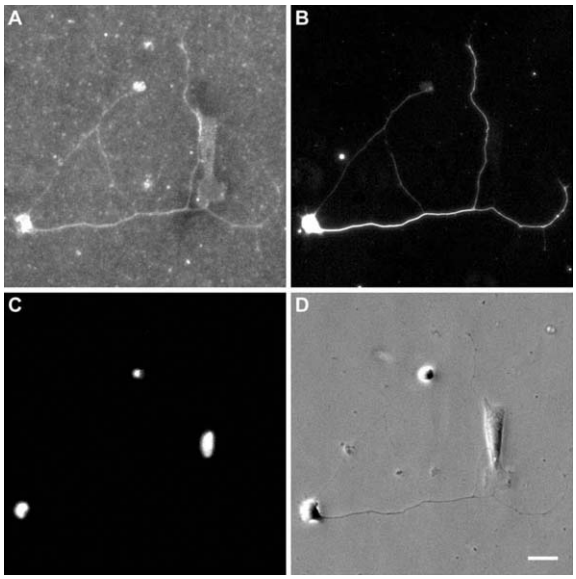

Fig. 3 DM-GRASP expression of DRG cells on DM-GRASP nanopatterns. (A) Neurons and mesenchymal cells express DM-GRASP with highest levels on the neuronal soma on 58 nm DM-GRASP patterns. Immunostaining of DM-GRASP results in detectable fluorescence levels of substrate DM-GRASP which range at about 60% of the fluorescence brightness displayed by the neurites. (B) Only neurons express tubulin β3, mesenchymal cells are negative. (C) DAPI stainings reveal that nuclei of neurons (left) are smaller and brighter than the elongated ones of mesenchymal cells (right). (D) Corresponding phase contrast micrograph. Scale bar: 50 μm.

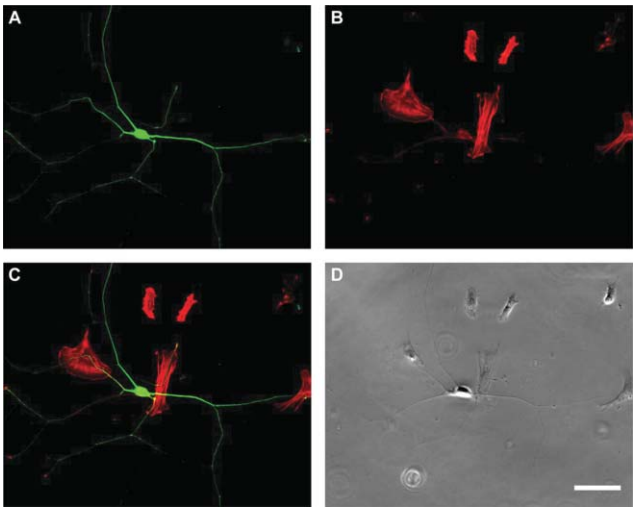

Fig. 4 Characterization of DRG cell type on DM-GRASP nanopatterns. (A) Neuronal marker tubulin β3 visualizes cells displaying the typical morphology of DRG neurons *in vitro* (rounded somata and several long and branched neurites) on 58 nm DM-GRASP pattern. (B) Staining of actin filaments by Cy3-conjugated phalloidin prominently labels the stress fibers of the mesenchymal cells (large, flat cells). (C) The merged picture reveals that the neuron is in contact with mesenchymal cells. The neurites cross mesenchymal cells indicating that this cellular substrate is not preferred to nanopatterned DM-GRASP substrate. (D) Corresponding phase contrast micrograph. Scale bar: 50 μm.

the cell adhesion mediating domain of fibronectin (RGD) pointing to distinct, cell type specific effects depending on the protein domain offered. Unfunctionalized gold dots do not mediate cell adhesion[1] indicating that putative binding of shed proteins to the gold dots does not take place or does not exert any effect on cell binding.

The number of cells bound to the 73 nm pattern is in the same range as on conventional substrates coated with poly-L-lysine (PLL) and PLL/laminin. Most of the cells on the nanopatterns show neuronal marker expression and the typical morphology of neurons (Fig. 3). Only about a quarter of the cells exhibit non-neuronal features, they represent the mesenchymal cells of the DRGs. To check whether DRG cells cultured on the edDM-GRASP nanopatterns express DM-GRASP, immunofluorescence labeling was performed. Neuronal as well as mesenchymal cells display DM-GRASP on the cell surface (Fig. 3B, D); thus both cell types are capable of direct, homophilic interactions with the substrate edDM-GRASP. On PLL and PLL/laminin substrates comparable numbers of neurons and mesenchymal cells are detected suggesting a more efficient adhesion of neurons to nanopatterned edDM-GRASP than to the conventional substrates. On nanopatterned edDM-GRASP, however, almost all neurons are in contact with mesenchymal cells (Fig. 4C), indicating that in addition to the edDM-GRASP substrate further stimuli might be needed for adhesion.

On edDM-GRASP nanopatterns, the majority of DRG cells is located in cell clusters (Table 1). Whereas a substantial amount of mesenchymal cells are able to adhere to the nanopatterned substrates as single cells (24% and 39% on 58 nm and 73 nm nanopatterns, respectively), only a very small number of neurons are without cellular contact (3% and 6% on 58 nm and 73 nm nanopatterns, respectively). On narrow-spaced edDM-GRASP, about four times more clusters are formed; moreover, large clusters are only found on this substrate and never on the wide-spaced one. Only a minor fraction of the cells in the clusters is mesenchymal (19% and 20% on 58 and 73 nm patterns, respectively); these cells are located at the bottom in contact with the substrate and might be responsible for the primary adhesion. DRG neurons are able to adhere directly to conventional substrates such as PLL or PLL/laminin, where they are mainly found as single cells (91% and 75%, respectively). On these substrates, similar fractions of mesenchymal cells are not in contact with other cells (88% and 74%, respectively). The high binding rate of single cells, indicating efficient adhesive properties of these substrates, might be partially due to shed proteins bound to the unpassivated surface. Together, narrow-spaced edDM-GRASP is a more efficient cell culture substrate for DRG cells than the conventional ones tested. Compared to the wide-spaced edDM-GRASP nanopatterns, five times more neurons and four times more mesenchymal cells adhere to the narrow-spaced nanopatterns. Neurons might, however, depend on mesenchymal cells for efficient adhesion and differentiation.

On 58 nm edDM-GRASP nanopatterns, DRG neurons, *in vivo* connecting body periphery and central nervous system with their neurites, form neurites ($\geqslant$20 μm). This is only extremely rarely observed on 73 nm pattern (160 and 7 neurites per 10 mm^2, respectively) indicating that the wider-spaced pattern is insufficient for neurite growth. On the narrow-spaced edDM-GRASP nanopattern, neurite bundles emerging from clusters are also detected (134 bundles per 10 mm^2); on wide-spaced nanopatterns

no bundles at all are formed. Preferential growth of neurites on other neurites (resulting in bundling, *i.e.* fasciculation) points to moderate growth supporting properties of the nanopatterned substrate. On PLL and PLL/laminin, neurite bundles are not found; these cultures only contain single neurites (33 and 243 neurites per 10 mm^2, respectively) formed by single neurons. On DM-GRASP nanopatterns, in contrast, single neurons are not able to send out neurites, thus neurite formation appears to depend on cell contact (Fig. 4). Immunofluorescence stainings reveal that the neurite-forming neurons, which are labeled by neuron-specific marker tubulin β3 (Fig. 4A), are always in contact with mesenchymal cell(s), which exhibit the typical morphology of fibroblasts, *e.g.* stress fibers, as visualized by actin staining (Fig. 4B). Once a neurite is formed, however, it is able to elongate over considerable distances on the narrow-spaced DM-GRASP substrate without any cellular contact (Fig. 4C, D) indicating that this nanopattern is sufficient to support but not to initiate neurite growth.

Gold dot nanopatterns provide several advantages compared to other nanopatterned structures such as those generated by a synthetic polymer-linking method.[8] (1) Proteins are presented in an exactly defined, uniform spacing. The accuracy of the patterned nanodots can be readily controlled using scanning electron microscopy. Functionalisation of each dot with histidine-tagged protein was demonstrated by atomic force microscopy.[9] (2) Only one (or very few) DM-GRASP molecule(s) can bind per nanodot due to the size of the gold dot (5 nm) and the dimensions of its extracellular domain (average width of Ig-like domains of CAMs: 4.5 nm).[10] For the two cell adhesion molecules agrin and N-cadherin binding of one or two proteins was demonstrated for 99% of the dots;[9] nanopatterns thus efficiently prevent the presentation of clustered proteins. (3) Presentation of edDM-GRASP in the same orientation as in the plasma membrane, *i.e.* the amino-terminal domain directed towards the opposing cell surface, is achieved by attachment of the histidine-tag to the carboxyl-terminal end of DM-GRASP's extracellular domain. It has been shown for two IgSF-CAMs that for their cellular impact (adhesion, neurite growth) simple coating is not sufficient and the amino-carboxyl terminal orientation as in the plasma membrane is crucial.[11,12]

These unique features of the nanopattern technique open up new, intriguing possibilities for the presentation of domains of integral membrane proteins in cell biological assays. CAMs coated conventionally on glass coverslips, in contrast, lack any physiological orientation. Thus, they do not allow unequivocal discrimination between *trans*- and *cis*-interactions; DM-GRASP and L1 *e.g.* are both known to be capable of both parallel and anti-parallel homophilic interactions.[13–15] Expression of a CAM in cell monolayers serving as substrates ensures a nearly physiological CAM presentation, is, however, hampered by an unknown degree of cluster formation and a multitude of unknown interaction partners present in the plasma membrane of the monolayer cells. Also protein-coated glass coverslips present a range of non-characterized proteins shed by the cells attached to them, in particular when cultured for several days as necessary *e.g.* for neurite outgrowth studies. Our nanopatterned substrates avoid such problems since they are equipped with a highly effective passivation layer as indicated by the fact that no cells at all adhere in non-structured substrate. Self assembling monolayers (SAMs) can also provide an effective passivation and amino-carboxyl terminal orientation; spacing (including prevention of substrate clusters) and concentration of the offered protein, however, cannot be tightly controlled

Already a small difference in DM-GRASP's spacing is able to affect the cellular response; it is noteworthy in this context that it is not possible to differentiate whether the observed cellular effects are caused by the change in lateral spacing (see below) or in net surface concentration (density) since both parameters interdepend. Whereas DM-GRASP offered as a 58 nm nanopattern is an efficient substrate for the adhesion of neuronal and mesenchymal cells, an increase in nanodot distance by 25% (which corresponds to a reduction in DM-GRASP-dot density of 32%) clearly decreases adhesion. The 58 and 73 nm substrates contain 280 and 190 nanodots μm^{-2}, respectively, which corresponds to approx. 50 000 and 35 000 nanodots on the substrate attachment side (177 μm^2) of a neuron (approx. diameter 15 μm). The DM-GRASP density on the cell surface is not known; for other IgSF-CAMs (NCAM, L1, CD146) and non-IgSF-CAMs (EpCAM, L-selectin, integrin) densities are in the range of 10^5 and 10^6 molecules per cell.[16–21] If this density range was also the case for DM-GRASP, the number of DM-GRASP molecules on the cell surface would exceed the number of contacted nanodots about 2 to 30-fold. Since DM-GRASP is one of the less abundant IgSF-CAMs and staining intensities indicate only a 1.5 fold surplus (see Fig. 3A), the DM-GRASP nanopatterns mimic approximately physiological densities of this CAM. Thus, for both 58 and 73 nm patterns, it can be supposed that each substrate DM-GRASP molecule interacts with cellular DM-GRASP which is presumably recruited into the cell attachment side by diffusion-mediated trapping. Since DRG neurons express DM-GRASP and the number of DM-GRASP molecules in the plasma membrane does not appear to be a limiting factor, the number of dots can be assumed to correlate directly to the number of homophilic *trans*-interactions. This might be the underlying reason why an increase of only 47% in substrate DM-GRASP density can result in a five-fold increase in cell adhesion; the strong cellular response points to amplification mechanisms *i.e.* intracellular signalling (see below) rather than a mere increase in adhesion. In addition, there is evidence that DM-GRASP can *trans*-interact with L1 which is expressed by DRG neurons[22] thereby contributing to the excess number of cellular binding partners; the heterophilic *trans*-interaction of DM-GRASP, however, appears to be clearly weaker than the homophilic one.[23]

Given a 2 to 30-fold excess of DM-GRASP in the cell membrane, about 50–97% of the DM-GRASP molecules are not directly engaged in the binding to the nanodots. These DM-GRASP molecules, however, are likely to join the *trans*-interacting ones to form clusters (by *cis*-interactions). It was demonstrated for NCAM and L1 that cluster formation is required for IgSF-CAM function;[24,25] there is evidence for three to six molecules per cluster, as found for L1, NCAM and tenascin.[14,26–28] A crucial prerequisite for the capacity of L1 to *cis*-interact and recruit other L1 molecules to form a cluster is the refolding of the extracellular domain induced by *trans*-interaction.[14] If this was the case also for DM-GRASP, clusters would only be formed in contact with DM-GRASP dots; the nanopattern would therefore determine the pattern and number of DM-GRASP clusters. Even if a high excess of DM-GRASP in the plasma membrane existed, those

DM-GRASP molecules not engaged in clusters would be inactive which would explain the relatively strong difference in cellular responses elicited by the narrow- and wide-spaced nanopatterns.

A comparable increase in adhesion (4 to 5-fold) has been found for fibroblasts and osteoblasts on RGD functionalized nanopatterns spaced 58 nm/73 nm.[1,29] RGD peptides *trans*-interact with integral membrane proteins, the integrins, which are crucial components of focal adhesions (multi-molecular complexes mediating adhesion and signalling). Interestingly, for both families, integrins and IgSF-CAMs, the same threshold distance (58 nm) cannot be exceeded without loss of cellular functions. It is therefore conceivable that the dimensions of proteins which link cluster components such as cortical cytoskeletal linker molecules might be critical, in particular ankyrin (25 nm) and talin (51 nm), known to bind to IgSF-CAMs and integrins, respectively;[30] also peripheral and integral membrane proteins might act as linking proteins. In this scenario, the lateral spacing of the offered CAM can be assumed to play a more critical role than its net density. Also the spreading range of the intracellular signalling elicited by the integral membrane proteins in a cluster bound to a nanodot could be limiting; to trigger a cellular response, the signalling might have to exceed a certain threshold level which is only reached on denser nanopatterns in the zones where the spreading signalling overlaps.[31] On the other hand, distances below 58 nm do not result in enhancement of elicited cellular responses. When DM-GRASP is offered as a 38 nm nanopattern, attachment of neuronal and non-neuronal cells as well as neurite extension does not differ from the effects found on 58 nm nanopatterns. This is in accordance with experiments employing 58 and 28 nm RGD nanopatterns for osteoblasts (Arnold *et al.*, 2004[1]) or the cell adhesion molecule agrin for neuronal cells[32] where no differences were observed.

Whether offered as a 58 or 73 nm pattern, DM-GRASP provides only insufficient support for neurons as they are only rarely found adhering singly to the nanopatterned substrate. The close apposition to a mesenchymal cell appears to be crucial for neurite formation since it is not observed for neurons without such a contact. Conceivably, in conventional cultures neurite outgrowth promoting stimuli are provided by proteins deposited on the unpassivated glass surface whereas in nanopattern cultures direct contact to mesenchymal cells supplying these signals is necessary as deposition is not possible. Neurites with lengths up to several hundred micrometres comparable to those in standard cultures, however, can be extended on the narrow-patterned DM-GRASP without cellular contact, demonstrating that DM-GRASP is sufficient to promote neurite elongation. Neurite growth not only depends on adhesion, which appears to be weak on DM-GRASP nanopatterns compared to standard substrates, but also on the activation of signalling pathways, changes in protein expression, and redistribution of proteins, in particular components of the cortical cytoskeleton.[33–35] Some of these cytoskeletal proteins are directly linked to IgSF-CAMs or are targets of their signalling, such as ankyrin, spectrin, actin, and tubulin. The short cytoplasmic domain of DM-GRASP does not contain any signalling motifs nor clear cytoskeleton interaction sites; DM-GRASP is therefore likely to recruit other proteins mediating these functions. It remains to be elucidated which components constitute the probably multi-molecular clusters induced by DM-GRASP *trans*-interaction and how they co-operate. In this context, nanopatterned substrates are superior tools, allowing both the control of proteins involved and the dimensions of their interactions.

Acknowledgements

We are grateful to H. Tanaka (Kumamoto University, Honjo, Japan) for the kind gift of chicken DM-GRASP cDNA. We thank M. Zieher-Lorenz, S. Bergmann, and C. Brandel for excellent technical support and H. X. Avci for stimulating discussions. The work was supported by the German Research Foundation (DFG) (SFB 488, GRK791), the Alfried Krupp von Bohlen und Halbach Foundation and the Landesstiftung Baden-Württemberg.

Notes and references

1 M. Arnold, E. A. Cavalcanti-Adam, R. Glass, J. Blummel, W. Eck, M. Kantlehner, H. Kessler and J. P. Spatz, *ChemPhysChem*, 2004, **5**, 383–388.
2 R. Glass, M. Möller and J. P. Spatz, *Nanotechnology*, 2003, **14**, 1153–1160.
3 F. R. Burns, S. von Kannen, L. Guy, J. A. Raper, J. Kamholz and S. Chang, *Neuron*, 1991, **7**, 209–220.
4 H. Tanaka, T. Matsui, A. Agata, M. Tomura, I. Kubota, K. C. McFarland, B. Kohr, A. Lee, H. S. Phillips and D. L. Shelton, *Neuron*, 1991, **7**, 535–545.
5 A. P. DeBernardo and S. Chang, *Dev. Biol.*, 1995, **169**, 65–75.
6 H. X. Avci, P. Zelina, K. Thelen and G. E. Pollerberg, *Dev. Biol.*, 2004, **271**, 291–305.
7 J. A. Weiner, S. J. Koo, S. Nicolas, S. Fraboulet, S. L. Pfaff, O. Pourquie and J. R. Sanes, *Mol. Cell. Neurosci.*, 2004, **27**, 59–69.
8 G. Maheshwari, G. Brown, D. A. Lauffenburger, A. Wells and L. G. Griffith, *J. Cell Sci.*, 2003, **113**(Pt 10), 1677–1686.
9 T. Wolfram, F. Belz, T. Schoen and J. P. Spatz, *Biointerphases*, 2007, **2**, 44–48.
10 C. Chothia and E. Y. Jones, *Annu. Rev. Biochem.*, 1997, **66**, 823–862.
11 E. J. Williams, B. Mittal, F. S. Walsh and P. Doherty, *J. Cell Sci.*, 1995, **108**, 3523–3530.
12 A. Buchstaller, S. Kunz, P. Berger, B. Kunz, U. Ziegler, C. Rader and P. Sonderegger, *J. Cell Biol.*, 1996, **135**, 1593–1607.
13 L. C. van Kempen, J. M. Nelissen, W. G. Degen, R. Torensma, U. H. Weidle, H. P. Bloemers, C. G. Figdor and G. W. Swart, *J. Biol. Chem.*, 2001, **276**, 25783–25790.
14 S. Silletti, F. Mei, D. Sheppard and A. M. Montgomery, *J. Cell Biol.*, 2000, **149**, 1485–1502.
15 K. Thelen, V. Kedar, A. K. Panicker, R. S. Schmid, B. R. Midkiff and P. F. Maness, *J. Neurosci.*, 2002, **22**, 4918–4931.
16 O. Nybroe, D. Linnemann and E. Bock, *J. Neurochem.*, 1989, **53**, 1372–1378.
17 M. Scherer, M. Heller and M. Schachner, *Eur. J. Neurosci.*, 1992, **4**, 554–562.
18 N. Bardin, F. Anfosso, J. M. Masse, E. Cramer, F. Sabatier, A. Le Bivic, J. Sampol and F. Dignat-George, *Blood*, 2001, **98**, 3677–3684.
19 S. Honda, J. J. Campbell, D. P. Andrew, B. Engelhardt, B. A. Butcher, R. A. Warnock, R. D. Ye and E. C. Butcher, *J. Immunol.*, 1994, **152**, 4026–4035.
20 B. M. Stockton, G. Cheng, N. Manjunath, B. Ardman and U. H. von Andrian, *Immunity*, 1998, **8**, 373–381.
21 S. K. Akiyama and K. M. Yamada, *J. Biol. Chem.*, 1985, **260**, 4492–4500.
22 A. P. DeBernardo and S. Chang, *J. Cell Biol.*, 1996, **133**, 657–666.
23 H. Avci and G. E. Pollerberg, unpublished results.
24 R. Sadoul, M. Hirn, H. Deagostini-Bazin, G. Rougon and C. Goridis, *Nature*, 1983, **304**, 347–349.
25 R. S. Schmid, W. M. Pruitt and P. F. Maness, *J. Neurosci.*, 2000, **20**, 4177–4188.
26 A. K. Hall and U. Rutishauser, *J. Cell Biol.*, 1987, **104**, 1579–1586.

27 J. W. Becker, H. P. Erickson, S. Hoffman, B. A. Cunningham and G. M. Edelman, *Proc. Natl. Acad. Sci. U. S. A.*, 1989, **86**, 1088–1092.
28 H. P. Erickson and J. L. Inglesias, *Nature*, 1984, **311**, 267–269.
29 E. A. Cavalcanti-Adam, A. Micoulet, J. Blummel, J. Auernheimer, H. Kessler and J. P. Spatz, *Eur. J. Cell Biol.*, 2006, **85**, 219–224.
30 D. L. Scott, G. Diez and W. H. Goldmann, *Theor. Biol. Med. Modell.*, 2006, **3**, 17.
31 A. R. Reynolds, C. Tischer, P. J. Verveer, O. Rocks and P. I. Bastiaens, *Nat. Cell Biol.*, 2003, **5**, 447–453.
32 T. Wolfram, unpublished results.
33 J. S. da Silva and C. G. Dotti, *Nat. Rev. Neurosci.*, 2002, **3**, 694–704.
34 T. Tojima and E. Ito, *Prog. Neurobiol.*, 2004, **72**, 183–193.
35 A. K. Panicker, M. Buhusi, K. Thelen and P. F. Maness, *Front. Biosci.*, 2003, **8**, d900–911.