ADVANCED BIOLOGY
www.advanced-bio.com

Screening Arrays of Laminin Peptides on Modified Cellulose for Promotion of Adhesion of Primary Endothelial and Neural Precursor Cells

*Richard Wetzel, Sandra Hauser, Weilin Lin, Peggy Berg, Carsten Werner, Jens Pietzsch, Gerd Kempermann, and Yixin Zhang**

Neural precursor cells (NPC) are primary cells intensively used in the context of research on adult neurogenesis and modeling of neuronal development in health and diseased states. Substrates that can facilitate NPC adhesion will be very useful for culturing these cells. Due to the presence of laminin in basal lamina as well as their involvement in differentiation, migration, and adhesion of many types of cells, surfaces modified with laminin-derived peptides are focused upon and compared with the widely used fibronectin-derived Arg-Gly-Asp (RGD) peptides. An array of 46 peptides is synthesized on cellulose paper (SPOT) to identify laminin-derived peptides that promote short-term adhesion of murine NPC and human primary endothelial cells. Various previously reported peptide sequences are re-evaluated in this work. Initial adhesion experiments show NPC preferred several laminin-derived peptides by up to 5-time higher cell numbers, compared to the well-known promiscuous integrin binding RGD peptide. Importantly, screening of cell adhesion has revealed a synergetic effect of filamentous matrix, peptide sequence, surface property, ligand density, and the dynamic process of NPC adhesion.

Cultures of primary and stem cells are fundamental tools for cell biology and medical research as well as for clinical applications.[1] Biomaterials specifically tailored for different cell types hold great promise for recapitulating stem cell niches and thus supporting the cultures of particular sensitive cells.[2–4]

Biomaterials can be prepared from natural substances such as proteins or extracts of the extracellular matrix (ECM) with Matrigel being a most frequently applied product.[5,6] Alternatively, synthetic materials can also be employed.[7–12] While proteins or ECM extracts can enable effective cell adhesion and most faithfully mimic the cellular microenvironments, synthetic materials offer more defined substrates with low batch-to-batch variation facilitating their applications especially for clinical purposes.[13,14]

Many primary and stem cells are anchorage-dependent cell types and require a certain degree of adhesion to a solid culture support to ensure cell survival, growth, and development in vitro.[10,15–17] To enable cell adhesion, synthetic materials can be modified with peptides ligands, which are specific adhesion sequences derived from ECM proteins. The first reported and most frequently used sequence Arg-Gly-Asp (RGD) has been identified by Ruoslahti and colleagues in 1984.[18,19] However, the RGD-based biomaterials are neither universal, nor cell type-specific substrates.[19,20] In the past decades, libraries of polymers and peptide-modified substrates have been

Dr. R. Wetzel, Dr. W. Lin, P. Berg, Prof. Y. Zhang
B CUBE Center for Molecular Bioengineering
Technische Universität Dresden
Tatzberg 41, Dresden 01307, Germany
E-mail: yixin.zhang1@tu-dresden.de

Dr. S. Hauser, Prof. J. Pietzsch
Department of Radiopharmaceutical and Chemical Biology
Helmholtz-Zentrum Dresden-Rossendorf
Institute of Radiopharmaceutical Cancer Research
Bautzner Landstraße 400, Dresden 01328, Germany

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/adbi.201900303.

DOI: 10.1002/adbi.201900303

Prof. C. Werner
Leibniz Institute of Polymer Research Dresden
Institute of Biofunctional Polymer Materials/
Max Bergmann Center of Biomaterials
Hohe Str. 6, Dresden 01069, Germany

Prof. J. Pietzsch
Faculty of Chemistry and Food Chemistry, School of Science
Technische Universität Dresden
Dresden 01062, Germany

Prof. G. Kempermann
German Center for Neurodegenerative Diseases (DZNE)
Tatzberg 41, Dresden 01307, Germany

Prof. G. Kempermann
CRTD—Center for Regenerative Therapies Dresden
Technische Universität Dresden
Fetscherstraße 105, Dresden 01307, Germany

created and screened to promote cell attachment for a large variety of cell types like cancer cell lines,[21,22] mesenchymal stromal cells,[23] embryonic stem cells,[24–26] induced pluripotent stem cells,[27,28] hepatic cells,[29] or microglial.[30]

Neural precursor cells (NPC) are primary cells intensively studied in the context of research on adult neurogenesis and modeling of neuronal development in health and diseased states.[31,32] NPC are strongly dependent on laminin as culture support.[33] Consequently, peptides from various laminin subtypes have been the focus of many researches.[34–39] It has been shown that NPC can be expanded on surfaces coated with laminin-derived IKVAV and YIGSR peptides.[40–42] However, reports and results differ from each other, as the peptides were displayed on different substrates,[43] and cell lines[35] as well as primary neurons[44] have been used in the different studies. In this study, we applied the SPOT technology as a medium-throughput array synthesis and screening approach to compare different NPC-adhesion peptide sequences.

SPOT is a variation of the classical solid phase peptide synthesis (SPPS) developed for synthesizing peptide arrays directly on cellulose paper for screening experiments such as epitope mapping.[45,46] By using this method, Toepert and colleagues screened more than 10 000 variants of a 38-mer WW protein domain with different dye-labeled proline-rich peptides.[47] Cellulose has further been successfully applied to directly screening materials in cell culture. Whitesides and colleagues have developed a multilayer paper-based assay. Cancer cells were cultured within droplets of Matrigel spotted on cellulose sheets, which could be stacked to reassemble 3D tissue for drug testing.[48,49] Deiss et al. generated an array of 96 peptides on a Teflon-patterned paper using a flow-through synthesis method and applied breast cancer cells to validate their method.[21] Kaur and co-workers have developed a peptide array-based on SPOT and applied this method for screening up to 70 peptides originated from p160 or EGFR for their cell binding capacities.[22,50] Cellulose has also been approved for culturing induced pluripotent stem cells.[51] Therewith, SPOT technology can offer an efficient strategy to prepare peptide libraries for screening cell adhesion peptides.[21,52] The peptide synthesis on PEG-amine-modified cellulose offers the advantage of generating high peptide density of up to 10 μmol cm^{-2} (400 nmol cm^{-2} in this study[53] exceeding surface concentration realizable on glass (e.g., <10 pmol cm^{-2} for a streptavidin coated surface).[26] With SPOT, peptide densities can be tuned and included as variable in the synthesis and screening.

In the present study, we evaluated 46 reported peptides originated from Fibronectin, Laminin, as well as Cadherins for their potency to support adhesion of NPC using SPOT technology. As reference and setup control, primary endothelial cells have been used. Endothelial cells such as human umbilical vein endothelial cells (HUVEC) or human dermal microvascular endothelial cells (HDMEC) have high affinity to fibronectin coated surface.[54–56] Therefore, these cells recognize and adhere to various RGD-modified materials. In contrast, NPC were expected to prefer laminin-derived sequences.

We aimed to probe the selectivity of peptide spots with cells in direct comparison based on their functional properties (e.g., sequence, ligand density, and surface charge). The peptides were synthesized on cellulose membranes and tested by submerging into cell suspension. Cells were incubated for 3 or 24 h before being subjected to washing steps, fixation, staining, imaging, and finally automated cell counting (**Figure 1**A; Figure S1, Supporting Information)

The peptides were synthesized on amino-PEG-functionalized cellulose paper using SPOT synthesis[45] (Figure S1A,B, Supporting Information). Acetylation reaction is used to cap unreacted N-terminal after each coupling cycle, in order to prevent the generation of deletions in resulting sequences, also resulting in acetylated amino-PEG chain in all peptide-free area (Figure S2, Supporting Information). Every spot had a diameter of 3 mm with a center-to-center distance of 6 mm and each array contained 4 × 9 peptide spots. 2–4 different peptide densities were used in the screening. The peptide synthesis on cellulose has been validated by adding a photo-cleavable linker at the C-terminal. The peptides could be released from cellulose membrane by photolysis and characterized by mass spectrometry (Table S1 and Figure S9, Supporting Information).

We further developed an imaging and image analysis protocol, to evaluate and compare cell number, size, and shape for each spot (Figure S1C, Supporting Information). The setup was validated by seeding NPC and HUVEC on the SPOT array of RGD peptides. The cells adhered specifically to the peptide spots and aligned along the cellulose fibers (Figure 1A). Moreover, neither HUVEC nor NPC adhered to the peptide-free area, creating a strong contrast between peptide spots and background of unmodified cellulose substrate. Cell counts on non-peptide modified areas were used as background adhesion for data analysis

Arrays of 3 different RGD peptides (R1–R3) in 4 different densities (Figure 1) were generated using SPOT synthesis. In addition, pre-synthesized cyclic-RGD peptide (R4) containing a cysteine residue was conjugated to maleimide-modified spots via Michael-type addition in 4 different densities (Figure 1B,C; Figure S3, Supporting Information). The strength of initial adhesion of NPC or HUVEC was evaluated by a standardized washing procedure after 3 h of incubation with cells, followed by fixation, staining, and imaging for nuclei and cell body (Figure 1B,C).

All four peptide types and densities promoted adhesion for HUVEC (Figure 1B). While neither sequence nor density of R1, R2, or R3-modified spots caused significant difference, the adhesion to R4-modified spots was shown to be concentration dependent, decreasing with decreasing ligand density. Surprisingly, the adhesion of NPC to R1, R2, and R3-modified cellulose increased upon lowering the ligand density, whereas the adhesion to R4-modifed cellulose decreased (Figure 1C). This effect can be explained by the dilution strategy: Dilution of the peptide concentration was achieved by mixing the first building block glycine-Fmoc with glycine-Boc at the given ratios (Figure S2, Supporting Information). After completion of the synthesis, the Boc group was removed in the TFA deprotection step. Lowering the densities of R1, R2, and R3 on cellulose spots is thus accompanied with the increase of free amino groups (PEG-Gly-NH_2), whereas the cellulose grafted with R4 does not possess free amino group (Figures S2 and S3, Supporting Information). The RGD peptides and PEG-Gly-NH_2 on cellulose could cause a synergistic effect on NPC adhesion. To demonstrate this, glycine-modified cellulose spots were generated (Figure S5A,

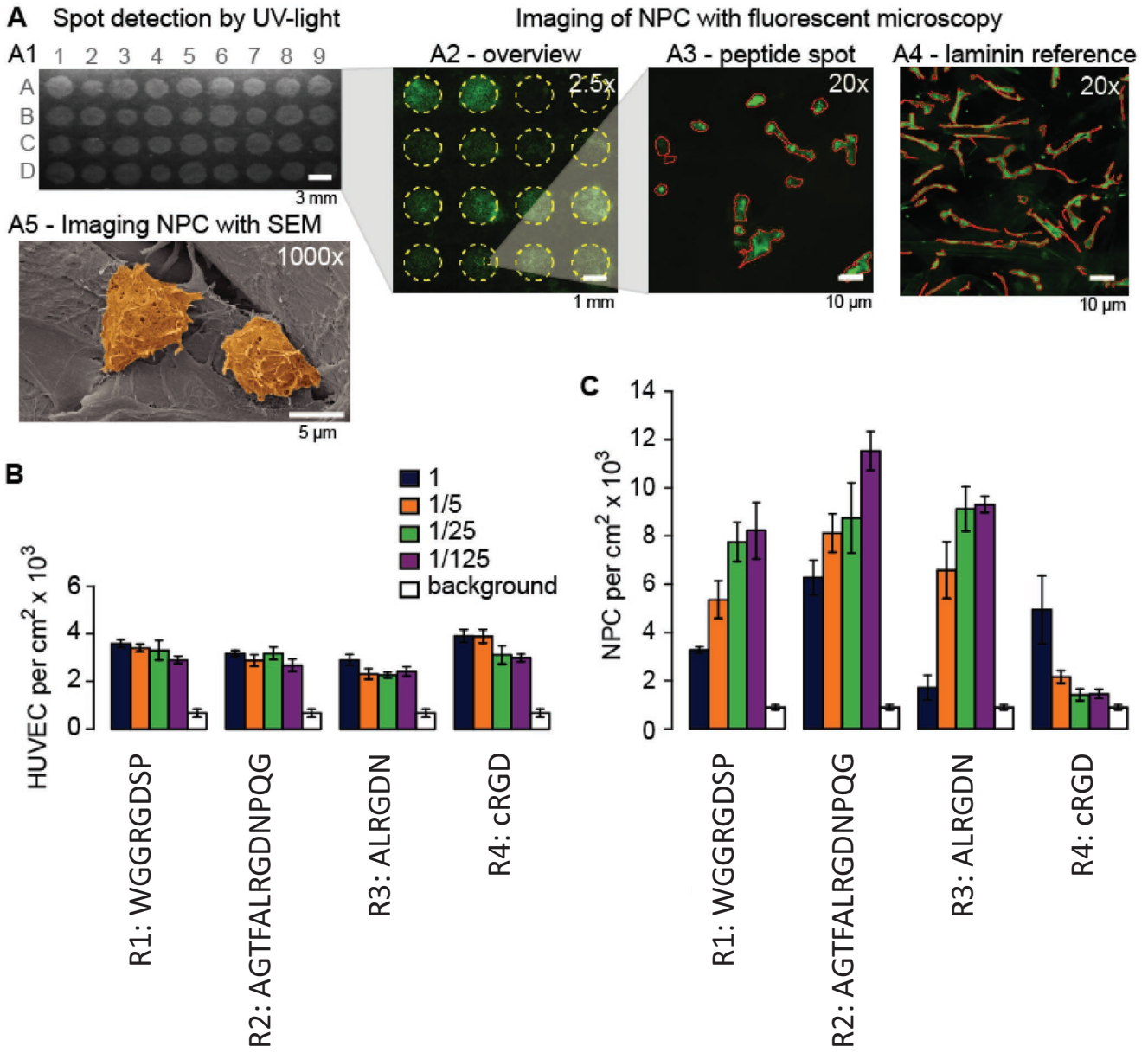

Figure 1. Peptide array on cellulose for cell-based screening. A1) Peptide spots can be visualized by UV light. A2–A4) NPC, attached to the peptide spots or laminin on the cellulose membrane, were stained for actin and imaged with fluorescent microscopy. A5) Scanning electron microscopy illustrated the location of the NPC on the cellulose fibers. B) HUVEC and C) NPC were seeded on the membrane functionalized with three different SPOT synthesized RGD peptides or grafted with cyclic RGD (cRGD) using thiol-maleimide addition, in four different densities. After 3 h of incubation surfaces were washed and cells were fixed. Cell counting was facilitated by automated image analysis of the fluorescent images. $n = 2$ biological with 3 technical replicates each. Mean ± SEM.

Supporting Information). The amino-rich matrix showed a moderate effect to promote NPC adhesion. In sum, different cell types responded differently to the adhesion ligands as well as ligand density and surface property. This suggests including not only different peptide sequences but also ligand densities as variables in a screening setup.

Endothelial cells interact with fibronectin-derived cell binding sequence RGD, and are also capable to interact with other adhesion peptides. We investigated the effect of 3 RGD peptides and 4 short non-RGD peptides (derived from various ECM proteins, Supporting Information), either as separate or as fused sequences on the adhesion of primary endothelial cells (**Figure 2**; Figure S4, Supporting Information). The adhesion of two different primary endothelial cells, HUVEC and HDMEC were investigated. All short non-RGD peptides have shown minor effects on promoting cell adhesion as compared to peptide-free area, while both endothelial cell types can attach strongly to the RGD peptide-modified spots.

We then investigated whether the non-RGD peptides would show a synergetic effect when fused with an RGD peptide

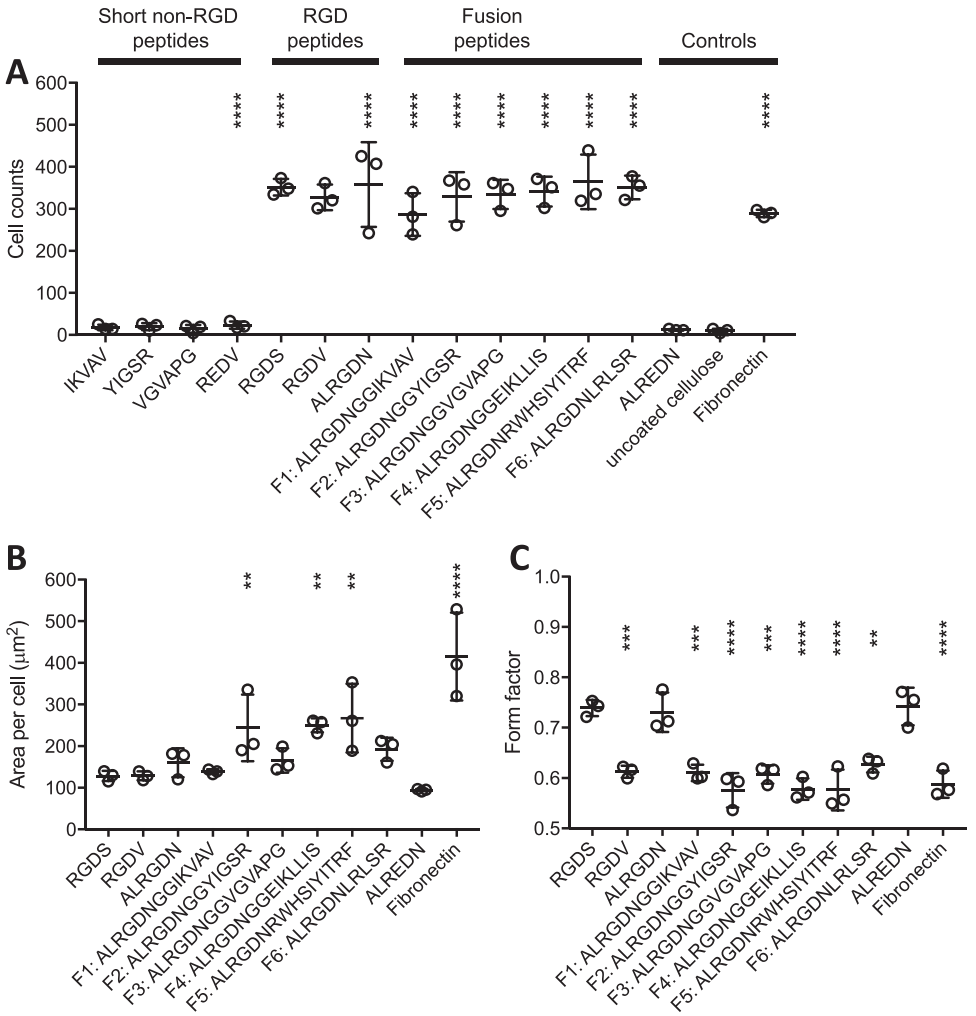

Figure 2. Adhesion test (3 h) for endothelial cells. HDMEC were seeded on cellulose array and fibronectin coated cell culture plastic. $n = 3$ technical replicates, mean ± SD. A) Analysis of cell numbers with one-way ANOVA $F(15, 32) = 48.3$ with $p < 0.0001$ and Dunnett's post-hoc test and comparison to negative control sequence ALREDN. B) Analysis of cell area with one-way ANOVA $F(15, 32) = 11.8$ with $p < 0.0001$ and Dunnett's post-hoc test and comparison to negative control sequence ALREDN. C) Analysis of form factor with 1 indicating a perfect circle and 0 a straight line. One-way ANOVA $F(15, 32) = 17.1$ with $p < 0.0001$ and Dunnett's post-hoc test and comparison to negative control sequence ALREDN. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

(Figure 2; Figure S4, Supporting Information). The six fused sequences did not provide increased numbers of adhered cells (Figure 2A; Figure S4B, Supporting Information), however, show remarkable effects on cell morphology (Figure 2B,C; Figure S4C,D, Supporting Information). While F2, F4, and F5 promoted spreading of HDMEC, all six fused sequences caused elongation of both HUVEC and HDMEC morphology with reduced form factors. It can be concluded that the fused sequences provide multiple biochemical cues to better mimic the basal lamina, resembling the cell morphology on fibronectin-coated surfaces.

Different from endothelial cells, adhesion of NPC was known to be highly dependent on laminin proteins, rather than the simple RGD ligand. Therefore, we have included a collection of 34 laminin-derived NPC adhesion peptides reported in literatures (L1–L19, L21–L35) in array design. Further, 9 peptides with Cadherin-derived sequences (C1–C9), two RGD references (R3), and a negative control (R5) have been included in the screening. Cadherins are adhesion proteins involved in cell–cell interaction and are important to modulate cellular behavior.[57]

High-density arrays of 46 peptides were synthesized (**Figure 3**). NPC were seeded on the arrays and tested for adhesion. While the RGD peptide R3 gave an adhesion of 2080 ± 226.6 cells per area, a remarkable difference among the 46 peptides was found. Laminin peptides L1 and L2 provided more than fivefold increased numbers of attached cells as compared to R3 (12 188 ± 576.3 and 11 617 ± 588.1, respectively). A few laminin peptides were less potent than R3. The negative control R5 did not show countable cells, demonstrating that cell adhesion was sequence specific. Interestingly, all cadherin peptides did not promote NPC adhesion either and were thus excluded from further analyses.

A low-density array (1/125) containing 29 laminin-derived sequences was synthesized to compare with the high-density array (Figure S5B, Supporting Information). The number of counted

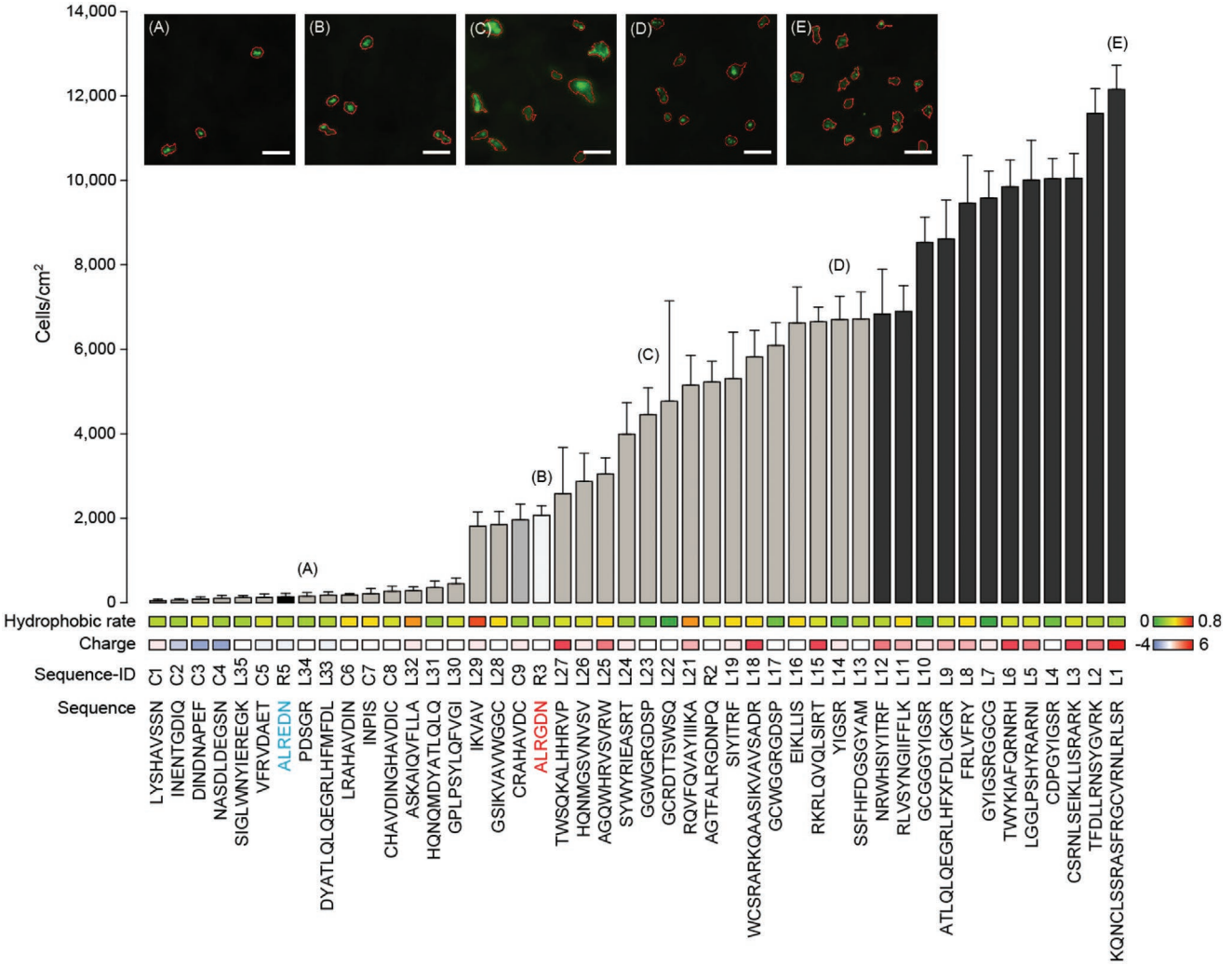

Figure 3. Screening of 46 peptides on cellulose for promotion of NPC adhesion. Cell counts are provided as cells per cm². A–E) Representative images of NPC on peptide spots. In addition to cell counts, the peptide sequences, the corresponding sequence IDs, as well as peptide net charge and hydrophobicity are provided. $N = 6–9$. Mean ± SEM. Black bars indicate differences to ALRGDN (white bar, sequence in red) by one-way ANOVA and Dunnett's post-hoc test at $p < 0.01$.

NPC was doubled on the low-density L22, L27, L28, and L29 spots as compared to the high-density spots, whereas the opposite effect has been observed for L3 and L4 peptides. Therefore, the synergistic effect of cell adhesive ligand and free amino group on NPC attachment is dependent on the peptide sequences. In general, the relatively weak adhesive sequences for NPC benefit more from the synergetic effects of the cellulose matrices.

To investigate the contribution of physical or chemical properties to the cell–material interaction, we analyzed the potential correlation between cell adhesion and net charge as well as hydrophobicity of peptides (Figure 3). While hydrophobicity did not correlate with cell adhesion, NPC showed a preference to neutral and positively charged peptides as adhesive substrates over negatively charged sequences (Figure 3; Figure S6, Supporting Information). We have found that laminin peptides can provide superior adhesion properties for NPC than the RGD peptide. While different binding preferences have been found in the library of laminin sequences, loading density, and physical property of peptides can also affect NPC adhesion.

We then investigated whether the screening results could be confirmed by grafting peptides synthesized by conventional SPPS to cellulose matrix (**Figure 4**A). Five peptides, L1, L8, L16, R3, and negative control R5 were synthesized using SPPS. To achieve covalent coupling to the maleimide functionalized cellulose, a cysteine residue was added to the C-terminals of sequences, while cysteine residues in the original sequences were replaced by methionine residues. Mass spectroscopy confirmed the correctness of the synthesized sequences (Figure S10, Supporting Information). It is important to note that the grafting method cannot achieve the high ligand density as SPOT synthesis. L1, L8, and L16 modified surfaces have shown enhanced adhesion to NPC, as compared with the RGD peptide. Although the cell adhesion is relatively lower on the grafted surfaces, the selectivity is in good agreement with the screening array (Figure 3) generated by SPOT. In comparison

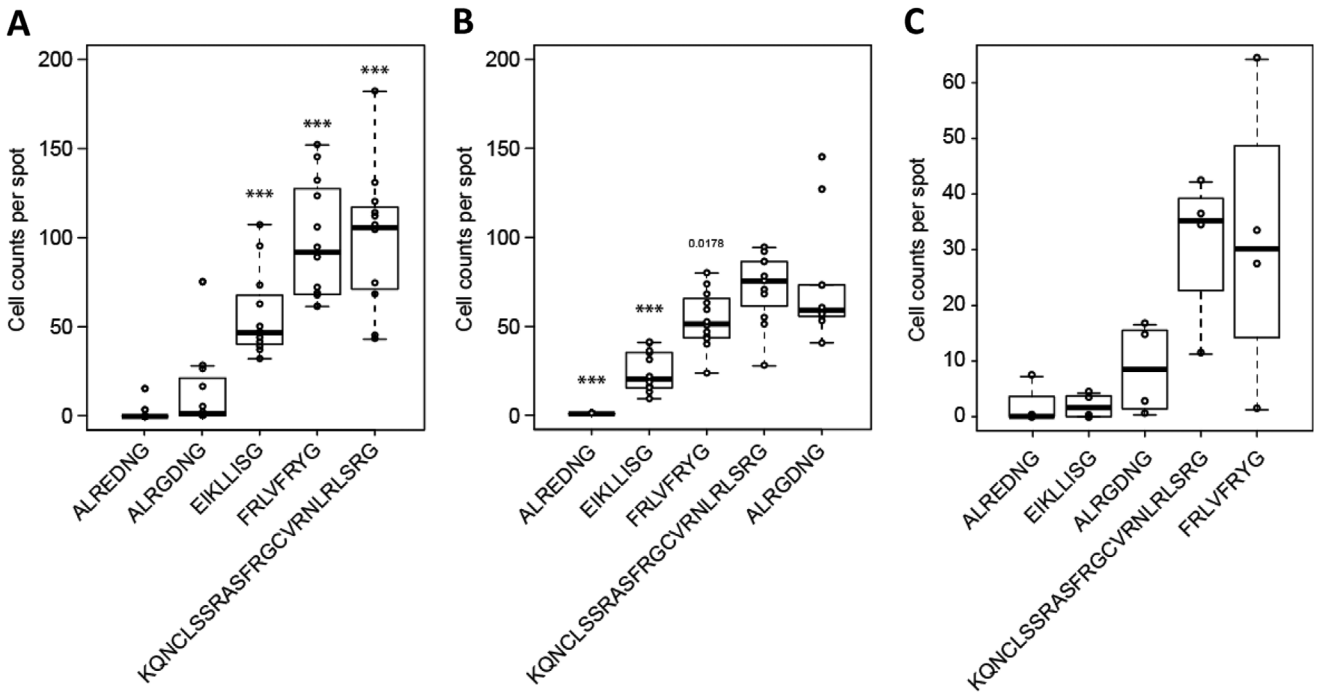

Figure 4. Analysis of NPC adhesion on peptide-grafted substrates. SPPS synthesized peptides were grafted to A,B) cellulose and C) PEMA-functionalized glass. NPC were seeded on the peptide arrays of 3 selected candidate peptides and 2 control sequences. ALREDNG was chosen as negative control and ALRGDNG as positive control. NPC were analyzed for cell numbers 3 h (A,C) and 24 h (B) post seeding. $N = 3$. Whiskers indicate the 2nd percentile and the 98th percentile. One-way ANOVA with Dunnett's post-hoc test comparing to ALREDNG, $^{***}p < 0.001$.

with the grafting approach, the SPOT approach is more time-efficient and flexible, generating materials with high peptide density, thus particularly suitable for primary screening.

The screening described above focused on the effective initial adhesion within 3 h after seeding. However, optimal conditions for cellular reorganization could differ from the initial adhesion tests. To investigate this relationship, we have seeded and incubated the cells over the time course of 24 h on cellulose paper grafted with L1, L8, L16, R3, or R5 peptides. Interestingly, remarkable changes were unveiled in cell number and morphology (Figure S7, Supporting Information). The RGD peptide R3 exhibited weak interaction with NPC after 3 h incubation in comparison to the laminin-derived peptides. After 24 h, R3, L1, and L8 modified celluloses have shown similar attachment to NPC (Figure 4B). Furthermore, the NPC showed elongated and stretched phenotype as indicated by a smaller form factor and larger area per cell on R3 (Figure S7, Supporting Information). This result underlines that the adhesion of NPC to cellulose materials is a dynamic process with different ligands contributing in different types of interaction over time. While laminin peptides enable effective adhesion in initial adhesion tests, RGD sequence promoted enhanced spreading of the cells.

We then investigated the application of these identified laminin peptides to other type of matrix (Figure 4C). Glass slides were coated with polyethylene maleic-anhydride copolymer (PEMA) and the functionalization with maleimide enables coupling of peptides by Michael-type addition. The grafting process was monitored by quartz crystal microbalance and ligand density can be tuned by varying concentration of Cys-containing peptide (Figure S8, Supporting Information). The L1 and L8 grafted PEMA surfaces have shown higher adhesion to NPC as compared to the RGD peptide, which is in line with the observed preferences on cellulose. However, the overall cell numbers were remarkably lower than on the corresponding cellulose surfaces. NPC adhered poorly to L16-grafted PEMA surface. These results indicate that the high surface concentrations possible on cellulose and its fibrous structure contribute to the adhesion of NPC on the SPOT array. While the peptide-modified cellulose surfaces would have great potential to improve the culturing condition of NPC, grafting the same peptides to other polymer surface such as PEMA has shown limited use in this study of NPC culture.

In this report, we screened SPOT array for the property to promote NPC adhesion, to re-evaluate various previously reported peptides sequences and to develop cellulose-based materials for NPC culture. The candidate matrices were selected by their adhesion-promoting properties and confirmed by post-screening validation. Through this systematic screening, we confirmed several laminin peptides outperforming RGD in a short-term adhesion assay. Interestingly, NPC showed an increased spreading on the RGD reference after 24 h of culture indicating differential requirements for adhesion and cellular reorganization of these particular cells. In the future, long-term culture of NPC will be investigated on these cellulose-based materials, while the technology will also be applied to support adhesion and guidance of differentiation of stem cells.

Experimental Section

Details of the materials and experimental methods are provided as Supporting Information.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

The authors thank Juliane Drichel, Milauscha Grimmer, Monique Marx (IPF) and Silke White (Imaging Platform, DZNE) as well as Catharina Knöfel and Johanna Wodtke (HZDR) for technical assistance. The authors further thank Petra Lennig of the Molecular Synthesis facility and Marc Gentzel of the Molecular Analysis–Mass Spectrometry facility at the CMCB, which was kindly supported by grants of the European Union Regional Development (ERDF/EFRE, #100232736), the German Federal Ministry of Education and Research (BMBF, #03Z2ES1, #03Z22EB1), and the German Research Foundation (DFG, #INST 269/731-1 FUGG). R.W. and G.K. were financially supported by the German Center for Neurodegenerative Diseases (DZNE). C.W. and J. P. are thankful to the DFG for supporting this work within the Collaborative Research Center Transregio 67 "Functional Biomaterials for Controlling Healing Processes in Bone und Skin—From Material Science to Clinical Application" (CRC/TRR 67/3; # 59307082). S.H. and J.P. also thank the Helmholtz Association for funding a part of this work through the Helmholtz Cross-Program Initiative "Technology and Medicine—Adaptive Systems". The authors thank Dejan Hušman for proofreading the manuscript.

Open access funding enabled and organized by Projekt DEAL.

Conflict of Interest

R.W. and Y.Z. are co-founders of denovoMATRIX GmbH, which develops and manufactures cell culture coatings.

Author Contributions

R.W., S.H., J.P., G.K., and Y.Z. designed the experiments. R.W. and Y.Z. wrote the manuscript. P.B. synthesized all peptides, R.W. and S.H. conducted cell culture, and R.W. analyzed the data. R.W., S.H., J.P., Y.Z., C.W., and G.K. discussed the results.

Keywords

endothelial cells, laminin peptides, neural precursor cells, peptide array, SPOT synthesis

Received: December 20, 2019
Revised: October 29, 2020
Published online: January 14, 2021

[1] M. P. Lutolf, H. M. Blau, *Adv. Mater.* **2009**, *21*, 3255.
[2] C. W. Chang, Y. Hwang, D. Brafman, T. Hagan, C. Phung, S. Varghese, *Biomaterials* **2013**, *34*, 912.
[3] J. W. Lambshead, L. Meagher, J. Goodwin, T. Labonne, E. Ng, A. Elefanty, E. Stanley, C. M. O'Brien, A. L. Laslett, *Sci. Rep.* **2018**, *8*, 701.
[4] C. M. Madl, S. C. Heilshorn, H. M. Blau, *Nature* **2018**, *557*, 335.
[5] M. C. Kibbey, *J. Tissue Cult. Methods* **1994**, *16*, 227.
[6] H. K. Kleinman, M. L. McGarvey, J. R. Hassell, V. L. Star, F. B. Cannon, G. W. Laurie, G. R. Martin, *Biochemistry* **1986**, *25*, 312.
[7] K. Saha, E. F. Irwin, J. Kozhukh, D. V. Schaffer, K. E. Healy, *J. Biomed. Mater. Res.* **2007**, *81*, 240.
[8] G. P. Raeber, M. P. Lutolf, J. A. Hubbell, *Biophys. J.* **2005**, *89*, 1374.
[9] Z. Melkoumian, J. L. Weber, D. M. Weber, A. G. Fadeev, Y. Zhou, P. Dolley-Sonneville, J. Yang, L. Qiu, C. A. Priest, C. Shogbon, A. W. Martin, J. Nelson, P. West, J. P. Beltzer, S. Pal, R. Brandenberger, *Nat. Biotechnol.* **2010**, *28*, 606.
[10] K. Thamm, K. Möbus, R. Towers, S. Segeletz, R. Wetzel, M. Bornhäuser, Y. Zhang, M. Wobus, *Adv. Biosyst.* **2020**, *4*, 2000008.
[11] L. G. Villa-Diaz, H. Nandivada, J. Ding, N. C. Nogueira-De-Souza, P. H. Krebsbach, K. S. O'Shea, J. Lahann, G. D. Smith, *Nat. Biotechnol.* **2010**, *28*, 581.
[12] R. Wieduwild, R. Wetzel, D. Husman, S. Bauer, I. El-Sayed, S. Duin, P. Murawala, A. K. Thomas, M. Wobus, M. Bornhäuser, Y. Zhang, *Adv. Mater.* **2018**, *30*, 1706100.
[13] E. A. Aisenbrey, W. L. Murphy, *Nat. Rev. Mater.* **2020**, *5*, 539.
[14] C. S. Hughes, L. M. Postovit, G. A. Lajoie, *Proteomics* **2010**, *10*, 1886.
[15] S. Rodin, L. Antonsson, C. Niaudet, O. E. Simonson, E. Salmela, E. M. Hansson, A. Domogatskaya, Z. Xiao, P. Damdimopoulou, M. Sheikhi, J. Inzunza, A. S. Nilsson, D. Baker, R. Kuiper, Y. Sun, E. Blennow, M. Nordenskjöld, K. H. Grinnemo, J. Kere, C. Betsholtz, O. Hovatta, K. Tryggvason, *Nat. Commun.* **2014**, *5*, 3195.
[16] P. J. Dolley-Sonneville, L. E. Romeo, Z. K. Melkoumian, *PLoS One* **2013**, *8*, e70263.
[17] S. Dånmark, A. Finne-Wistrand, A. C. Albertsson, M. Patarroyo, K. Mustafa, *Biomed. Mater.* **2012**, *7*, 035011.
[18] M. D. Pierschbacher, E. Ruoslahti, *Nature* **1984**, *309*, 30.
[19] N. Huettner, T. R. Dargaville, A. Forget, *Trends Biotechnol.* **2018**, *36*, 372.
[20] E. Ruoslahti, *Annu. Rev. Cell Dev. Biol.* **1996**, *12*, 697.
[21] F. Deiss, W. L. Matochko, N. Govindasamy, E. Y. Lin, R. Derda, *Angew. Chem., Int. Ed.* **2014**, *53*, 6374.
[22] K. Kaur, S. Ahmed, R. Soudy, S. Azmi, in *Peptide Libraries*, Humana Press, New York, NY **2015**, p. 239.
[23] S. Gobaa, S. Hoehnel, M. Roccio, A. Negro, S. Kobel, M. P. Lutolf, *Nat. Methods* **2011**, *8*, 949.
[24] A. Ranga, S. Gobaa, Y. Okawa, K. Mosiewicz, A. Negro, M. P. Lutolf, *Nat. Commun.* **2014**, *5*, 4324.
[25] D. G. Anderson, S. Levenberg, R. Langer, *Nat. Biotechnol.* **2004**, *22*, 863.
[26] J. R. Klim, L. Li, P. J. Wrighton, M. S. Piekarczyk, L. L. Kiessling, *Nat. Methods* **2010**, *7*, 989.
[27] A. D. Celiz, J. G. W. Smith, A. K. Patel, R. Langer, D. G. Anderson, D. A. Barrett, L. E. Young, M. C. Davies, C. Denning, M. R. Alexander, *Biomater. Sci.* **2014**, *2*, 1604.
[28] P. J. Wrighton, J. R. Klim, B. A. Hernandez, C. H. Koonce, T. J. Kamp, L. L. Kiessling, *Proc. Natl. Acad. Sci. U. S. A* **2014**, *111*, 18126.
[29] C. J. Flaim, S. Chien, S. N. Bhatia, *Nat. Methods* **2005**, *2*, 119.
[30] M. Sarwat, D. C. Surrao, N. Huettner, J. A. St John, T. R. Dargaville, A. Forget, *Biomed. Mater.* **2020**, *15*, 055033.
[31] H. Babu, J. H. Claasen, S. Kannan, A. E. Rünker, T. Palmer, G. Kempermann, *Front. Neurosci.* **2011**, *5*, 89.
[32] F. Ehret, S. Vogler, G. Kempermann, *Stem Cell Res.* **2015**, *15*, 514.
[33] P. E. Hall, J. D. Lathia, M. A. Caldwell, C. Ffrench-Constant, *BMC Neurosci.* **2008**, *9*, 71.
[34] V. M. Freitas, V. F. Vilas-Boas, D. C. Pimenta, V. Loureiro, M. A. Juliano, M. R. Carvalho, J. J. V. Pinheiro, A. C. M. Camargo, A. S. Moriscot, M. P. Hoffman, R. G. Jaeger, *Am. J. Pathol.* **2007**, *171*, 124.
[35] K. Hozumi, A. Sasaki, Y. Yamada, D. Otagiri, K. Kobayashi, C. Fujimori, F. Katagiri, Y. Kikkawa, M. Nomizu, *Biomaterials* **2012**, *33*, 4241.
[36] F. Katagiri, M. Ishikawa, Y. Yamada, K. Hozumi, Y. Kikkawa, M. Nomizu, *Arch. Biochem. Biophys.* **2012**, *521*, 32.

[37] M. Makino, I. Okazaki, S. Kasai, N. Nishi, M. Bougaeva, B. S. Weeks, A. Otaka, P. K. Nielsen, Y. Yamada, M. Nomizu, *Exp. Cell Res.* **2002**, *277*, 95.
[38] P. K. Nielsen, Y. S. Gho, M. P. Hoffman, H. Watanabe, M. Makino, M. Nomizu, Y. Yamada, *J. Biol. Chem.* **2000**, *275*, 14517.
[39] M. Nomizu, K. Yamamura, H. K. Kleinman, Y. Yamada, *Cancer Res.* **1993**, *53*, 3459.
[40] Š. Kubinová, D. Horák, N. Kozubenko, V. Vaněček, V. Proks, J. Price, G. Cocks, E. Syková, *Biomaterials* **2010**, *31*, 5966.
[41] X. Li, X. Liu, B. Josey, C. J. Chou, Y. Tan, N. Zhang, X. Wen, *Stem Cells Transl. Med.* **2014**, *3*, 662.
[42] D. Thid, K. Holm, P. S. Eriksson, J. Ekeroth, B. Kasemo, J. Gold, *J. Biomed. Mater. Res.* **2008**, *84*, 940.
[43] B. Ananthanarayanan, L. Little, D. V. Schaffer, K. E. Healy, M. Tirrell, *Biomaterials* **2010**, *31*, 8706.
[44] T. T. Yu, M. S. Shoichet, *Biomaterials* **2005**, *26*, 1507.
[45] R. Frank, *Tetrahedron* **1992**, *48*, 9217.
[46] K. Hilpert, D. F. H. Winkler, R. E. W. Hancock, *Nat. Protoc.* **2007**, *2*, 1333.
[47] F. Toepert, T. Knaute, S. Guffler, J. R. Pirés, T. Matzdorf, H. Oschkinat, J. Schneider-Mergener, *Angew. Chem., Int. Ed.* **2003**, *42*, 1136.
[48] R. Derda, A. Laromaine, A. Mammoto, S. K. Y. Tang, T. Mammoto, D. E. Ingber, G. M. Whitesides, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 18457.
[49] F. Deiss, A. Mazzeo, E. Hong, D. E. Ingber, R. Derda, G. M. Whitesides, *Anal. Chem.* **2013**, *85*, 8085.
[50] H. Hossein-Nejad-Ariani, E. Althagafi, K. Kaur, *Sci. Rep.* **2019**, *9*, 2723.
[51] L. Wang, C. Xu, Y. Zhu, Y. Yu, N. Sun, X. Zhang, K. Feng, J. Qin, *Lab Chip* **2015**, *15*, 4283.
[52] H. E. Blackwell, *Curr. Opin. Chem. Biol.* **2006**, *10*, 203.
[53] M. D. Bowman, M. M. Jacobson, B. G. Pujanauski, H. E. Blackwell, *Tetrahedron* **2006**, *62*, 4715.
[54] B. Baudin, A. Bruneel, N. Bosselut, M. Vaubourdolle, *Nat. Protoc.* **2007**, *2*, 481.
[55] D. S. Grant, H. K. Kleinmann, G. R. Martin, *Ann. N. Y. Acad. Sci.* **1990**, *588*, 61.
[56] S. Hauser, F. Jung, J. Pietzsch, *Trends Biotechnol.* **2017**, *35*, 265.
[57] U. Tepass, K. Truong, D. Godt, M. Ikura, M. Peifer, *Nat. Rev. Mol. Cell Biol.* **2000**, *1*, 91.