Cell Adhesion

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Photocontrolled Cell Adhesion on a Surface Functionalized with a Caged Arginine-Glycine-Aspartate Peptide**

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Contact between cells and the extracellular matrix (ECM) is necessary for cell adhesion, growth, and migration, and is mediated by cell-surface receptors.[1] Binding between the ECM and the receptor results in adhesion of the cell to the ECM and the spreading of cells, and is critical for cell survival in vivo and in vitro.[2] Adherence to the surface of the culture dish is a prerequisite for successful cultivation of most cell lines. It is difficult to study binding and the subsequent signaling pathways kinetically because binding and cell adhesion occur spontaneously in culture dishes. Herein we describe a technique for manipulating cell adhesion by using a photoresponsive culture dish.

The method we developed involves an RGD (arginine-glycine-aspartate) peptide, which has been identified as a major integrin ligand motif in ECMs such as fibronectin and laminin,[3] and has been used to modify biomaterials to enhance cell adhesion.[4] We prepared a caged RGD peptide, the sequence of which (YAVTGRGDSPASS) is the longest conserved sequence in vertebrates ranging from teleosts to mammals but containing a nitrobenzyl group as a cage.[5] The 2-nitrobenzyl group was introduced at the amide bond between the Gly and Arg residues because this site is critical for biological activity.[6] The photoresponsive culture dish (Figure 1) was prepared by modifying a commercially available culture dish coated with poly-L-lysine (PLL) by using a bifunctional cross-linked polyethylene glycol (PEG) and the caged RGD peptide.

We used HeLa cells to study adhesion to the photoresponsive culture dish because they are typical of adhesive cells. The cells were plated and preincubated for 30 minutes and the dish then rinsed with phosphate-buffered saline (PBS). The cells, which did not adhere to the dish, were readily removed by gentle replacement of the culture medium with PBS (0 min in Figure S2 in the Supporting Information), thus showing that the photoresponsive culture dish was inactive towards cell adhesion when the peptide was in the caged form.

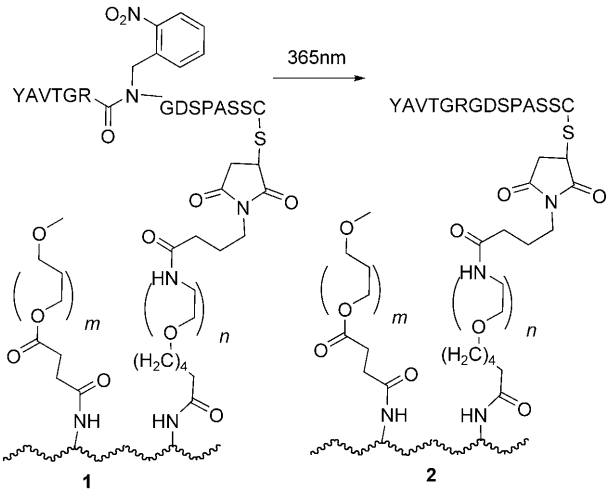

Figure 1. Photochemical reaction of a caged RGD peptide attached to a culture dish. The caged RGD peptide was linked to poly-L-lysine (depicted as a wavy line).

Irradiation of the photoresponsive culture dish with UV light converted it into a cell-adhesive state (see Figure S2 in the Supporting Information). The cells remained on the UV-irradiated dish after rinsing, but had spread and were flat, namely, the cells were adherent. The number of adherent cells correlated with the duration of irradiation of the dish.

The photoresponsiveness of the dish can be ascribed completely to the nitrobenzyl moiety, which can be removed by UV irradiation (Figure 1). HeLa cells adhered to the culture dishes coated with the RGD peptide, but did not adhere to culture dishes coated with PLL or a DGR peptide (see Figure S3 in the Supporting Information). There was no change in the adhesive properties of the culture dishes coated with the intact RGD peptide, PLL, or PEG upon irradiation with UV light. These control experiments show that the intact RGD peptidic moiety is responsible for cell adhesion in this system and that conversion of the photoresponsive culture dish into an adhesive state is caused by the release of the nitrobenzyl group from the cage. The correlation between the number of adherent cells and the duration of UV irradiation of the plate was not linear (see Figure S2 in the Supporting Information), which suggests that a threshold density of RGD moieties may be required for adhesion.[4,7]

Photolithography is an important component of tissue engineering and screening based on cell chips.[8] Spatially restricted cell adhesion was achieved by limiting the area of UV irradiation in the photoresponsive culture dish (Figure 2).

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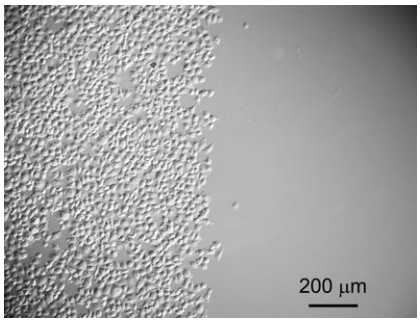

Figure 2. Spatial control of cell adhesion. The left half of the dish was irradiated during cell cultivation. The micrograph was recorded after washing and fixing.

After plating the cells, half of the dish was irradiated with UV light for 1 minute and the plate was then incubated for 20 minutes at 37 °C. After the dish had been rinsed with PBS, adherent cells were observed only on the half of the dish that had been exposed to irradiation. The cells had a flattened appearance. The UV irradiation did not damage the cells: they exhibited normal actin filaments and round nuclei after further cultivation (see Figure S4 in the Supporting Information).

Cell adhesion could have been triggered by the UV irradiation (Figure 3; see also Movie S4 in the Supporting Information). After the cells were plated on the photoresponsive culture dish, they were round and had no focal adhesions, as shown by their tendency to roll around when the dish was agitated (Figure 3b). Upon UV irradiation, most cells started spreading: many pseudopods were extended, the fringes of the cells enlarged, and the cell-height decreased (Figure 3a). The speed of cell spreading varied widely, presumably because of variation in the free areas between the cells and in the stages of the cell cycle (Figure 3c). The cells were irradiated with a tungsten lamp whilst simultaneously observing them by microscopy; exposure to this light source did not induce adhesion and there was no significant increase in the area covered by the cells (Figure 3b,d). This finding shows that cell adhesion triggered by UV irradiation can be analyzed using visible light. As most fluorescent probes such as fura-2 and green fluorescent protein (GFP) are excited by visible light, the photoresponsive culture dish should be compatible with fluorescence microscopy studies.

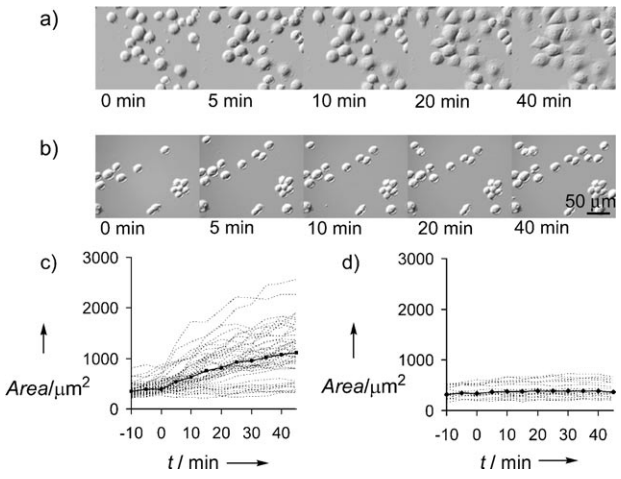

Figure 3. Temporal control of cell adhesion. Micrographs (a, b) and temporal changes in cell areas (c, d) are shown. The dish was irradiated with UV light just after recording the micrograph at 0 min (a, c). The results obtained in the absence of irradiation are shown in (b) and (d). Dotted lines indicate the areas of individual cells, and solid lines indicate the average area of the cells (c, d).

The events that occur after interaction between a cell and the ECM are rapid and intricate.[1] Several functional substrates are available for controlling the cell-adhesion responses to stimuli such as light,[8] heat,[9] and voltage.[10] Two strategies for phototriggering cell adhesion with cyclic-RGD peptides have been reported: photoswitching of the distance and orientation of the RGD peptides to the surface,[8g] and caging of the carbonyl group of Asp, which is required for binding to the MIDAS region of integrin.[8j] Our approach is similar to the latter approach, but is based on a different caging mechanism: the nitrobenzyl group was introduced as a cage at the amide bond of the Arg-Gly unit, which is also a critical moiety in the complex formed between the RGD peptide and integrin.[6] The chemical stability of this caging group should be superior to that of the ester.

The kinetics of the early stage of cell adhesion show that our photoresponsive culture dish has attributes not offered by other photoinduced cell-adhesion dishes. Although the substrates reported previously[8–10] can be used to control cell adhesion with high spatial resolution, to our knowledge there is no method that enables the analysis of cell adhesion with a temporal resolution of less than a minute. This is easily achieved under practical conditions by photocontrolling the cell adhesion, presumably because of 1) rapid and clean photocleavage of the nitrobenzyl group (see Figures S5 and S6 in the Supporting Information);[4] 2) stability of the caging group at the amide bond in aqueous solutions; 3) the use of a PEG linker with a high molecular weight, which, as predicted by del Campo and co-workers,[8j] precludes nonspecific adhesion between proteins and cells; and 4) the poly-L-lysine basal scaffold, which increases the surface density of the PEG linker and the RGD peptide. Our results show that the photoresponsive culture dish is a useful tool for maintaining non-adherent cells and triggering cell adhesion at a desired time in a specific area of the plate. Therefore, this technique would be useful for detailed analysis of certain aspects of the binding of cells to the ECM, such as cell spreading.[11] This technique can also be applied to other caged ligands such as the YIGSR motif of laminin.

In summary, we have developed a method that enables spatiotemporal photoregulation of cell adhesion by using a culture dish coated with a caged RGD peptide.

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[1] C. M. Nelson, J. Tien, *Curr. Opin. Biotechnol.* **2006**, *17*, 518 – 523.
[2] D. G. Stupack, D. A. Cheresh, *J. Cell Sci.* **2002**, *115*, 3729 – 3738.
[3] M. D. Pierschbacher, E. Ruoslahti, *Nature* **1984**, *309*, 30 – 33.
[4] U. Hersel, C. Dahmen, H. Kessler, *Biomaterials* **2003**, *24*, 4385.
[5] a) Y. Tatsu, T. Nishigaki, A. Darszon, N. Yumoto, *FEBS Lett.* **2002**, *525*, 20 – 24; b) C. D. Wood, T. Nishigaki, Y. Tatsu, N. Yumoto, S. A. Baba, M. Whitaker, A. Darszon, *Dev. Biol.* **2007**, *306*, 525 – 537.
[6] a) M. Dechantsreiter, E. Planker, B. Matha, E. Lohof, G. Holzemann, A. Jonczyk, S. L. Goodman, H. Kessler, *J. Med. Chem.* **1999**, *42*, 3033 – 3040; b) J. P. Xiong, T. Stehle, R. Zhang, A. Joachimiak, M. Frech, S. L. Goodman, M. A. Arnaout, *Science* **2002**, *296*, 151 – 155.
[7] C. Roberts, C. S. Chen, M. Mrksich, V. Martichonok, D. E. Ingber, G. M. Whitesides, *J. Am. Chem. Soc.* **1998**, *120*, 6548 – 6555.
[8] a) Y. Nakayama, M. Matsuda, *Macromolecules* **1996**, *29*, 8622 – 8630; b) Y. Nakayama, A. Furumoto, S. T. Matsuda, *Photochem. Photobiol.* **2003**, *77*, 480 – 486; c) Y. Luo, M. S. Shoichet, *Biomacromolecules* **2004**, *5*, 2315 – 2323; d) Y. Luo, M. S. Shoichet, *Nat. Mater.* **2004**, *3*, 249 – 253; e) J. Nakanishi, Y. Kikuchi, T. Takarada, H. Nakayama, K. Yamaguchi, M. Maeda, *J. Am. Chem. Soc.* **2004**, *126*, 16314 – 16315; f) J. Edahiro, K. Sumaru, Y. Tada, K. Ohi, T. Takagi, M. Kameda, T. Shinbo, T. Kanamori, Y. Yoshimi, *Biomacromolecules* **2005**, *6*, 970 – 974; g) J. Auernheimer, C. Dahmen, U. Hersel, A. Bausch, H. Kessler, *J. Am. Chem. Soc.* **2005**, *127*, 16107 – 16110; h) A. L. DeMond, T. Starr, M. L. Dustin, J. T. Groves, *J. Am. Chem. Soc.* **2006**, *128*, 15354 – 15355; i) J. Nakanishi, Y. Kikuchi, S. Inoue, K. Yamaguchi, T. Takarada, M. Maeda, *J. Am. Chem. Soc.* **2007**, *129*, 6694 – 6695; j) S. Petersen, J. M. Alonso, A. Specht, P. Duodu, M. Goeldner, A. del Campo, *Angew. Chem.* **2008**, *120*, 3236 – 3239; *Angew. Chem. Int. Ed.* **2008**, *47*, 3192 – 3195.
[9] R. A. Stile, K. E. Healy, *Biomacromolecules* **2001**, *2*, 185 – 194.
[10] Y. Li, B. Yuan, J. Hang, D. Han, S. Chen, F. Tian, X. Jiang, *Angew. Chem.* **2007**, *119*, 1112 – 1114; *Angew. Chem. Int. Ed.* **2007**, *46*, 1094 – 1096.
[11] G. Giannone, B. J. Dubin-Thaler, H. G. Dobereiner, N. Kieffer, A. R. Bresnick, M. P. Sheetz, *Cell* **2004**, *116*, 431 – 435.

