Cell–Surface Interactions

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Phototriggering of Cell Adhesion by Caged Cyclic RGD Peptides**

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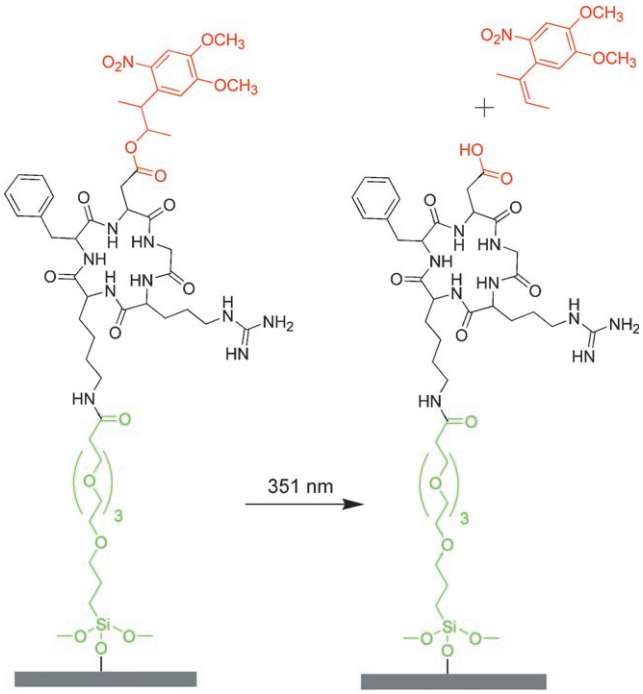
The controlled and selective adhesion of cells to surfaces is an important issue in cell biology and tissue engineering. Different strategies have been reported in which thermally,[1] photochemically,[2,3] and electrochemically[4] responsive surfaces and materials are used to manipulate cell adhesion. A more generic approach that would be suitable for any system, independent of its chemical constitution, would be advantageous. Such a strategy could not rely on material properties; instead, the molecular interactions involved in cell attachment must be controlled directly.

The design of a strategy to trigger the attachment event needs to consider the sensitivity of cells to most triggering sources (electric fields, chemical stimuli, pressure, and temperature jumps). Light of wavelength above 320 nm appears to be a convenient trigger, as its interaction with biomolecular species is negligible. Light-controlled cellular attachment requires the development of photosensitive molecules able to mediate cellular adhesion and whose activity changes upon irradiation. For this study, we selected the RGD cell-adhesive peptide, well known to promote integrin-mediated cell adhesion,[5,6] and modified it by introducing a photolabile caging group on the carboxylic acid side chain of the aspartic acid residue (Scheme 1). The presence of the caging group may cause steric hindrance, conformational constraint, or changes in the charge distribution of the peptide and thus prevent recognition of the peptide by the integrins. Light irradiation releases the cage from the peptide structure and restores the activity of the peptide to enable in situ site and temporal control of cell attachment. Cell-repellent surfaces modified with the caged peptide ("off" state) can become cell-adhesive ("on" state) upon irradiation with light of the appropriate wavelength and intensity.



Scheme 1. Chemical structure of cyclo[RGD(DMNPB)fK] (DMNPB in red) attached to the surface through the TEG linker (green). The caging group is released upon irradiation at 351 nm.

The selection of the caging position requires previous knowledge of the structural characteristics of the RGD–integrin binding site. In the particular case of the pentapeptide cyclo(-Arg-Gly-Asp-D-Phe-Val-) (cyclo(RGDfK)), a very active and selective ligand of integrin $\alpha_V\beta_3$,[7] it has been shown that the binding site involves two divalent cations, and that the aspartate unit acts as a ligand for one of them.[8,9] Therefore, we decided to introduce the caging group at this position. It is also known that the amino acid in the fifth position (Lys) does not have significant influence on the activity of the peptide.[7] The free amine group of the Lys residue has been used as anchoring position through which the peptide can be coupled to surfaces.[10]

3-(4,5-Dimethoxy-2-nitrophenyl)-2-butyl ester (DMNPB) was selected as the photolabile caging group ($\lambda_{max} = 346$ nm, $\varepsilon_{max} = 4100\,M^{-1}cm^{-1}$).[11] The caged Asp derivative DMNPB-Asp-Fmoc (Fmoc = 9-fluorenylmethoxycarbonyl) and the caged peptide cyclo[RGD(DMNPB)fK] were obtained and characterized as described in the Supporting Information.[11,12] Their UV spectra are shown in Figure 1.

The photolytic properties of the caged peptide in solution were then determined quantitatively. Upon exposure for 2 h to light of wavelength 364 nm in neutral buffered solution, up to 70% of cyclo[RGD(DMNPB)fK] disappeared, and up to 93% of the photolytic reaction product obtained was

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

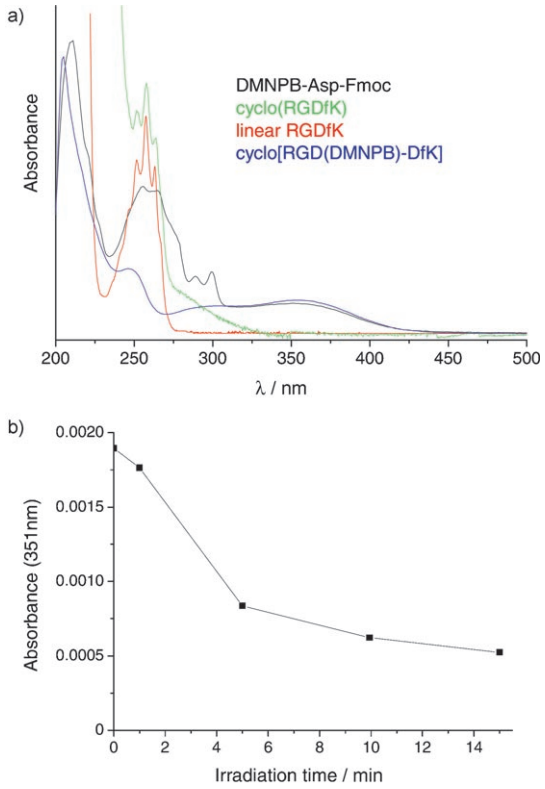

Figure 1. a) UV spectra of DMNPB-Asp-Fmoc, cyclo(RGDfK), linear RGDfK, and cyclo[RGD(DMNPB)fK] in solution. b) UV absorption measured at 351 nm for substrates modified with cyclo[RGD-(DMNPB)fK] after irradiation at 351 nm for different periods of time and washing. The decay in the absorption values corresponds to the cleavage of the chromophore from the surface during exposure to light.

identified as uncaged cyclo(RGDfK). A quantum yield of 0.34 was determined (see the Supporting Information).[13]

To test specific RGD-mediated integrin binding to surfaces, nonspecific interactions between the integrins or any other molecule at the cell membrane and the surface should be prevented. At the same time, the retention of protein activity and cell function in the surface-immobilized state is necessary. These requirements are fulfilled typically by using surface-modification agents that contain oligo(ethylene glycol) (OEG) spacers.[14] Therefore, we synthesized a bifunctional tetra(ethylene glycol) (TEG) linker containing a triethoxysilane moiety at one end for anchoring to the silica surface and an *N*-hydroxysuccinimide-activated carboxylic acid functionality at the other end for coupling to the free amine group of the Lys residue of the caged peptide.[15] The structure of the linker is shown in Scheme 1, and its synthesis is described in the Supporting Information.

Silica surfaces were modified with the linker by solution-phase silanization. Silane layers of up to 1.75 ± 0.05 nm in thickness were obtained, and a static contact angle of 40° was observed. The coupling of the peptide to the surface was monitored by UV/Vis spectroscopy on modified quartz substrates. The profile of the absorbance bands corresponding to the DMNPB chromophore and the peptide bonds appeared to be identical to the spectrum in solution. The contact angle of the surface increased by 3° upon peptide coupling to the linker. This value agrees with literature values for RGD-modified silicon wafers.[16] Ellipsometry measurements showed a small increase in the layer thickness, almost within the error range.

We examined the kinetics of the photolysis of DMNPB at the silica surface on the basis of the decay of the UV/Vis absorption of the peptide-modified substrates at 351 nm after different irradiation times (Figure 1b; see also the Supporting Information). Irradiation cleaves the DMNPB group from the surface layer. Subsequent washing removes the chromophore from the surface. According to the absorbance values, irradiation for 10 min at 351 nm cleaved 64% of the chromophores. Longer irradiation times did not cause a further significant decrease in absorption. We attribute the remaining absorption to DMNBP entrapped as a photoproduct within the surface layer and unable to be removed by washing.

The ability of the caged- and uncaged-RGD-modified surfaces to attach cells selectively through RGD–integrin recognition was tested in a cell-adhesion assay. Fibroblasts 3T3 were plated onto substrates modified with cyclo[RGD-(DMNPB)fK] before and after irradiation, and onto substrates modified only with the linker molecule as a negative control. We then analyzed the adhesion of fibroblasts by optical microscopy after different incubation times (3, 6, and 21 h) and counted the number of attached cells per unit area (Figure 2). A very small number of attached cells (ca. 2000 cells cm^{-2}) were observed for substrates modified only with the linker molecule after incubation for 6 h; this number increased to 5000 cells cm^{-2} after incubation for 21 h. This result confirms that the TEG surface prevents nonspecific cell adhesion. It is remarkable that the cell-repelling properties of the surface remain almost unchanged after incubation for 21 h in spite of the short length of the TEG spacer.

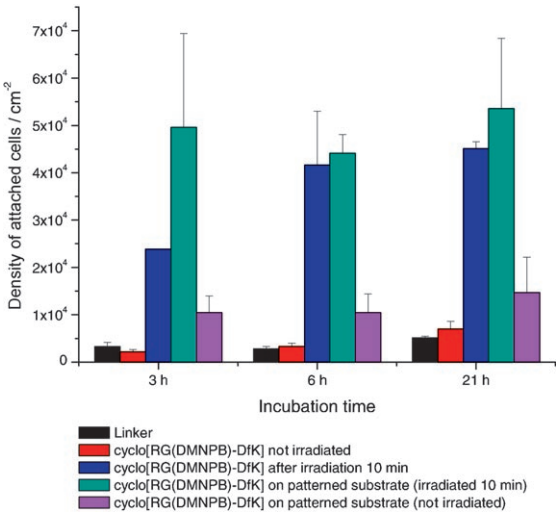

Figure 2. Cell density on surfaces modified with cyclo[RGD-(DMNPB)fK] before and after exposure to light of wavelength 351 nm for 10 min. Data are given for nonpatterned and patterned substrates. For comparison, data for surfaces modified only with the linker molecule are also shown.

The number of attached cells on the surfaces containing caged cyclo[RGD(DMNPB)fK] is similar to the number of cells attached to the substrate without the adhesive peptide, a result that indicates the lack of bioactivity of the caged RGD peptide. The number of cells attached to surfaces containing active cyclo(RGDfK) was approximately 11 times higher after incubation for 3 h and 13 times higher after incubation for 6 h. This result serves as evidence that irradiation removes the DMNPB cage and restores bioactivity. The maximum selectivity (difference in the number of cells attached to substrates modified with active RGD and substrates without active RGD) was observed after incubation for 6 h. Longer incubation times led to an increase in the number of cells attached to the substrate, but decreased selectivity. This decrease in selectivity may be a consequence of the adsorption of proteins from the medium on the surface. To improve the selectivity, more-repellent surface layers (e.g. the use of longer OEG chains) would be necessary.

These results show that cell attachment to surfaces can be inhibited efficiently by blocking the carboxylic acid side chain of the aspartic acid residue in the RGD motif. This conclusion is supported by the fact that cells attached to RGD-containing surfaces already show a high degree of spreading after 6 h, whereas cells attached to caged-RGD-containing surfaces need 21 h to start spreading. The use of the photosensitive DMNBP cage as a trigger for light-modulated cell adhesion provides much better results in terms of efficiency and selectivity than other reported photoactivation strategies based on the azobenzene unit.[17–19] However, the activation process is not reversible with the caged compounds.

We then tested the possibility of using cyclo[RGD-(DMNPB)fK] for in situ site-selective control of cell attachment. Cells were first plated on substrates modified with cyclo[RGD(DMNPB)fK], and the substrate was subsequently masked irradiated from the back side. Irradiation generated stripes of activated RGD on the surface. Figure 3 shows microscopic images of plated cells after different incubation times. The cells adhered preferentially to the irradiated regions, which contain active RGD, and made a cell pattern on the substrate. Cells on these samples were always oriented statistically; a negligible effect of the pattern on cell behavior was observed. Consequently, the results obtained can be attributed solely to the presence or absence of RGD.

A new caged peptide able to phototrigger cell attachment at surfaces has been developed. In its caged form, cyclo-[RGD(DMNPB)fK], the peptide did not show any integrin-binding activity. Upon activation with light, the peptide effectively mediated cell adhesion to surfaces with spatiotemporal definition. We envision numerous applications of such systems: Further studies are in progress towards the development of in situ patternable substrates for cell arrays, triggerable cell-adhesive coatings for artificial scaffolds in tissue engineering, and latent solution agents capable of inhibiting, or even reverting, cell attachment to surfaces after light activation.

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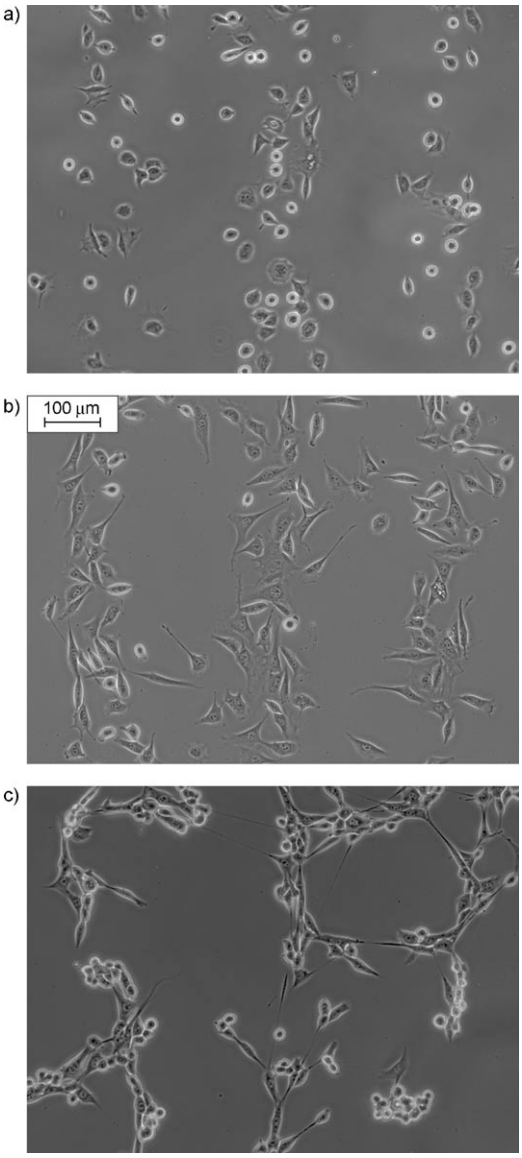

Figure 3. In situ triggering of cell attachment to substrates with cyclo[RGD(DMNPB)fK]. Cells were first plated onto substrates modified with the caged peptide and subsequently irradiated through a mask. The optical microscopy images show cells adhering preferentially to the irradiated regions (stripes of 100 μm in width), in which the peptide is in its uncaged form. The images show fibroblasts after incubation for 3 (a), 6 (b), and 21 h (c).

Keywords: cage compounds · cell adhesion · peptides · photolysis · responsive surfaces

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