Triggered Cell Release from Materials Using Bioadhesive Photocleavable Linkers

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The ability to trigger or turn "on" or "off" material properties with external stimuli in order to control biological responses is critically important to biotechnological and biomedical applications. One such application is the use of light to trigger cell adhesion to synthetic materials by controlling the presentation of the bioadhesive arginine-glycine-aspartic acid (RGD) oligopeptide. Successful strategies for photoactivation of cell adhesion include direct modification of the chemical structure of the RGD peptide with photoresponsive molecules,[1] using a light-triggered phase transition of a RGD-containing amphiphilic peptide,[2] and linking the RGD peptide to the surface using azobenzene units.[3] However, no light-based approaches are available to specifically promote cell adhesion to materials with the ability to precisely deactivate or detach the cells at later time points or spatial locations. This is a relevant issue in cell biology because cells need to be removed from the culture plate during culture and before application and in tissue engineering, i.e., cell sheet engineering therapies. In this article, we introduce a flexible and facile strategy for controlled light-triggered cell release from surfaces based on the use of photocleavable linkers to couple the RGD ligand to the surface. Upon light exposure, the linker is cleaved by means of a photolytic reaction,[4] thereby untethering the RGD peptide from the surface and releasing adhering cells. Our approach represents a more specific and controlled alternative to enzymatic digestion, temperature-driven changes in substrate hydrophobicity,[5] or electrochemical dissolution of polyelectrolyte coatings[6] or self-assembled monolayers (SAMs)[7] onto conductive substrates for cell release (see ref. [8] for a recent review on this topic). Our strategy is applicable to any material, provided that there is proper linker design, and provides direct control over the molecular interactions involved in cell adhesion.

The general strategy is presented in **Figure 1**. The photocleavable linker contains an intercalated 4,5-dialkoxy 1-(2-nitrophenyl)-ethyl photolabile group and can be attached to free amine groups at the surface of the material via a carbamate bond. A tetraethyleneglycol (TEG) spacer is included in the structure in order to provide a protein- and cell-repellent surface before attaching bioactive ligands. A bioactive ligand, such as biotin or RGD oligopeptide, can be attached to the surface by reacting with the free amine at the end of the TEG spacer (I). The biological ligand then mediates specific binding to the surface of a particular target, such as streptavidin for biotin or cells for RGD peptides (II). Upon light exposure, the chromophore is photocleaved (III) and the linker, along with its target, is effectively removed from the surface (IV). As with photolithographic techniques, full control over the spatiotemporal resolution of the release process is possible. By using a photomask for the illumination steps, patterns of active and nonactive sites can be previously generated, and immobilized species can be site-selectively detached in the last step.

The detailed synthesis of the linker is included in the Supporting Information. The linker was successfully reacted with amine-terminated substrates and the coupling reaction was monitored by UV spectroscopy on modified quartz substrates (where the characteristic bands of the chromophore were visible) and by ellipsometry on modified silicon wafers (an increase in the layer thickness of 1.4 nm was detected). The photocleavage of the linker from the surface was followed by UV spectroscopy after irradiating quartz substrates at λ_{max} = 350 nm for increasing times and washing with tetrahydrofurane and water. Exposure of *o*-nitrobenzyl derivatives to light induces an intramolecular redox reaction and cleavage of the cage from the surface (see scheme in **Figure 2**). Washing removed the photocleaved part of the molecule from the surface, and this was reflected in a significant decrease in absorbance in the UV spectrum, most visible around λ_{max} (Figure 2). The ratio between the absorbance pre- and post-irradiation at λ_{max} represents the amount of photocleaved linker upon exposure (conversion). After 8 min irradiation, no significant changes were visible in the UV spectrum with time, indicating that a maximum conversion of 70% was reached (inset in Figure 2). The residual absorbance indicates that part of the chromophore and/or photolytic by-products remained attached to the surface or entrapped by the surface layer.[9] The residual attached chromophore could be a potential limitation in the performance of the light-induced detachment step.

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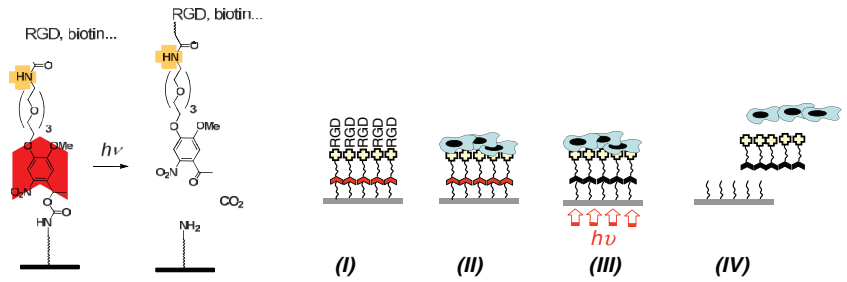
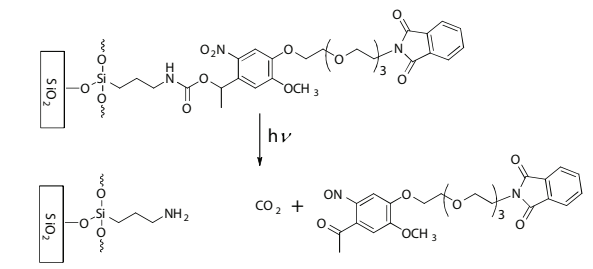


Figure 1. Left: Chemical structure of the photocleavable linker after reaction with the surface and the ligand before and after light exposure. Right: Working principle of phototriggered release, specifically for cell detachment from surfaces using photocleavable linkers with an intercalated cage.

In order to assess the ability of the photolabile moiety to mediate specific binding and detachment, we tested the attachment and controlled removal of the fluorophore AlexaFluor 647 succinimidyl ester and of streptavidin after functionalization with biotin. **Figure 3** and Figure A (Supporting Information) show schemes of the process. A substrate modified with the photosensitive linker was irradiated through a mask containing 100 µm wide chrome stripes separated by 200 µm wide gaps. Irradiation cleaved the linker from the exposed regions (Figure 3, I). The remaining linker molecules in the nonexposed areas were reacted with the fluorophore (Figure B, II, Supporting Information) or with *N*-(+)-biotin *N*-hydroxysuccinimide ester and then incubated with fluorescently labelled streptavidin (Figure 3, II). Figure 3A shows the fluorescent protein pattern observed through the microscope. The nonirradiated regions appeared bright, indicating preferential and site-controlled attachment of the protein. In a second irradiation step, the substrate was exposed through the same mask rotated 90°. After washing, fluorescence decreased in the irradiated regions (III and cross pattern in Figure 3B and Figure A, Supporting Information) as a consequence of the cleavage of the intercalated cage and removal of the attached specie (protein or fluorophore) from the surface. Low levels of residual fluorescence were observed in the irradiated regions due to uncleaved chromophore (up to 30%) remaining attached to the surface after full exposure, as anticipated from the photolysis studies (see conversion plot Figure 2). Negative controls were performed in order to prove that the loss of fluorescence was not due to bleaching of the fluorophore. No cross pattern was visible in the photobleached control. Taken together, these



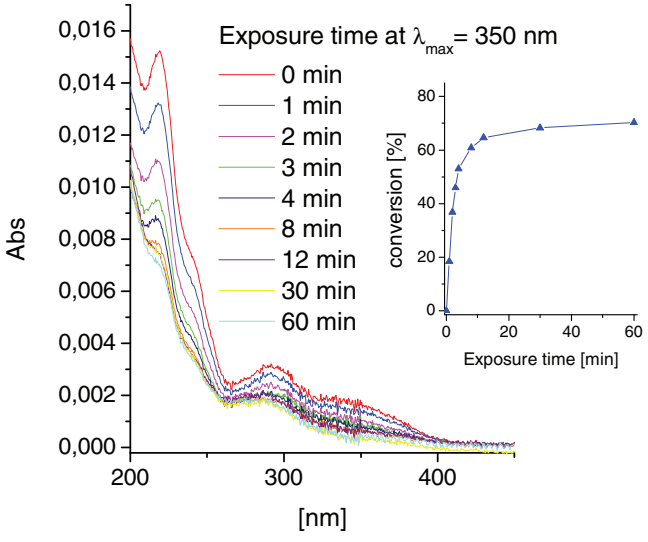


Figure 2. UV spectra of quartz substrates modified with the photosensitive linker after irradiation at 350 nm (0.6 mW cm^{-2}) for increasing exposure times and washing for removal of the photolysis products. Inset represents the photolytic conversion calculated from the decay in the absorbance.

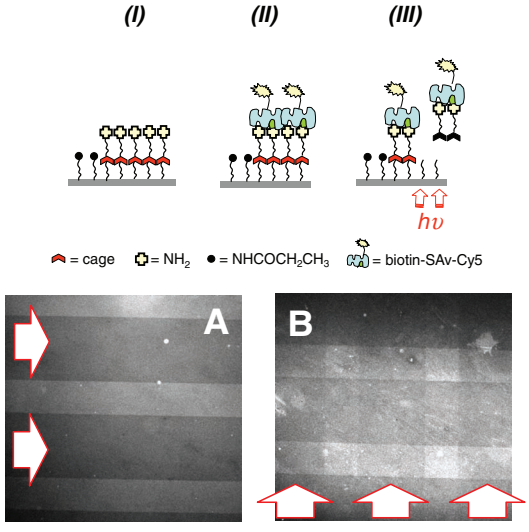


Figure 3. Phototriggered protein release and the possibility of site control. The scheme represents the coupling and irradiation steps. The fluorescent micropatterns correspond to A) step II (site-selective immobilization) and B) step III (site-selective release). The arrows indicate the exposed stripes (200 µm).

results demonstrate the potential of this approach to selectively attach and release molecules from surfaces with spatiotemporal control, in contrast to other work that requires additional blocking steps to prevent non-specific protein adsorption onto the surface.[10] The inclusion of the TEG segment in the linker provides an effective protein-repellent surface background.

We next examined the ability of photolabile linker to mediate precise cell attachment to and subsequent light-triggered detachment from surfaces via the presence or absence (via photocleavage) of RGD. For this purpose, mixed SAMs of amine- and methoxy-terminated thiols with oligo(ethylene glycol) spacers were used to modify gold-coated surfaces and obtain a cell-repellent surface. A non-adhesive background is crucial in order to guarantee only RGD-mediated cell attachment and minimize non-specific interactions of the cell with the surface. Cell experiments were performed on SAMs using a 2% surface concentration of the amine-terminated thiol to which the photolabile linker and cyclo(RGDfK) were attached. As a positive control, cyclo(RGDfK) was attached directly to the amine-terminated SAMs without the photolabile linker. Substrates were seeded with human umbilical vascular endothelial cells (HUVECs) for 2 h. No cell adhesion was observed on surfaces that did not present RGD peptide (**Figure 4**E). For surfaces modified with photocleavable linker and RGD, cells attached and spread, extending cellular processes (Figure 4A). Similar levels of spreading were observed on surfaces modified with photocleavable linker presenting RGD and surfaces modified directly with RGD (Figure 4C). This result demonstrates that the RGD peptide coupled to the photocleavable linker displays full bioadhesive activity. Importantly, control surfaces presenting cyclo(RADfK) as inactive peptide displayed minimal cell adhesion, confirming that adhesion to the surfaces is mediated by integrin cellular receptors (Figure 4F). Next, substrates were irradiated under conditions for photocleavage of the linker. On surfaces modified with photocleavable linker presenting RGD peptide, the cells rounded up and detached from the surface (Figure 4C). Over 85% of the original, adherent cells were removed from the surface by simple washing with saline, and the remaining cells displayed a round morphology, indicative of poor adhesion to the surface. We attribute the small fraction of remaining cells to incomplete cleavage of the photolabile linker as shown in the photolysis analysis. Importantly, cells remained attached and spread on the positive control substrates where the RGD was directly immobilized (without photolinker, Figure 4D), confirming that the cell release is associated with the cleavage of the linker and that the irradiation dose used in the experiments did not affect cell adhesion and spreading. In fact, cell staining with the Live/Dead kit showed no differences in viability between irradiated and not irradiated cell cultures under these exposure conditions (data not shown). These results demonstrate a flexible and facile strategy for the specific attachment of cells with the ability of subsequent controlled

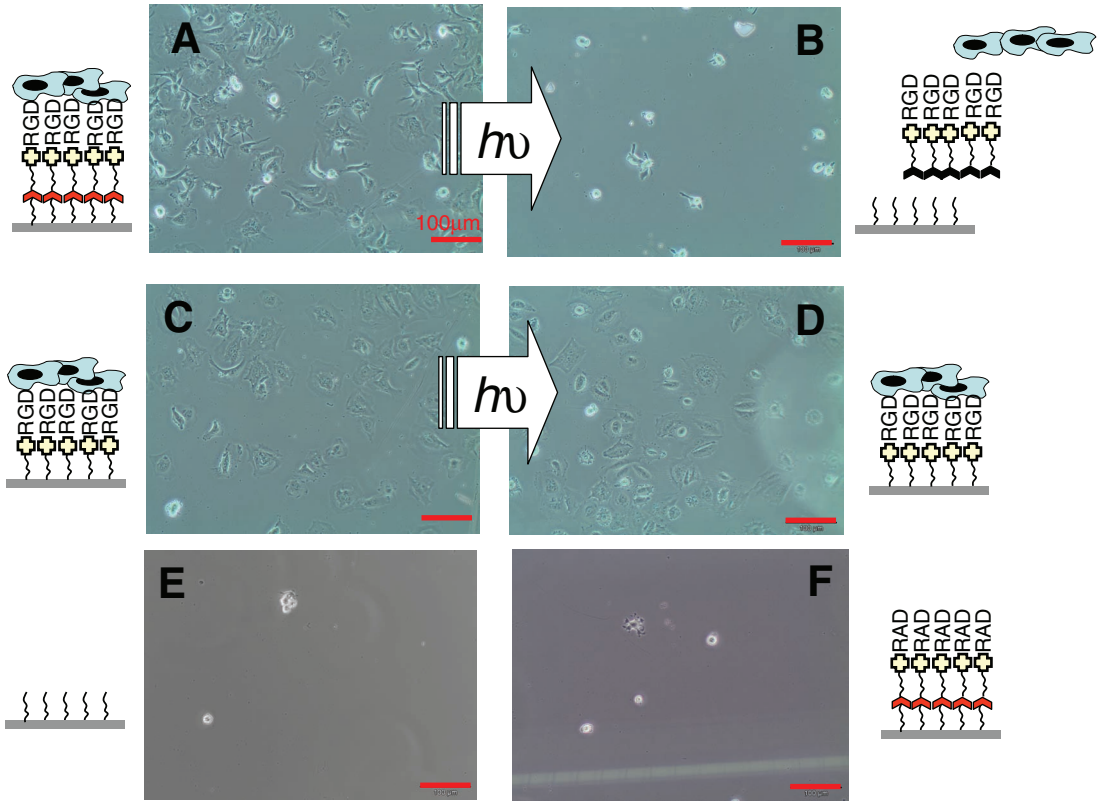


Figure 4. Microscopy images of HUVECs after 2 h incubation on RGD-modified substrates anchored to A) an amine-terminated SAM with the photosensitive linker and C) without the photosensitive linker (control experiment). In (B,D) the same substrates after irradiation are shown. E) HUVECs after a 2 h incubation on an amine-terminated SAM. F) HUVECs incubated for 2 h on cyclo(RADfK) non-adhesive peptide coupled to the amine-terminated SAM with the photosensitive linker. Scale bars represent 100 μm.

light-triggered release from surfaces. This strategy represents a more specific and controlled alternative to trypsination or to temperature-driven changes in substrate hydrophobicity,[5] or to the recently demonstrated cell release by addition of soluble RGD.[11] Reported cell release strategies relying on dissolution of the underlying electroactive coatings[6–8] or photodegradable gels[12] are material- and substrate-specific and less flexible than these photocleavable linkers. This approach is applicable to any material, provided that the linker is properly designed, and provides direct control over the molecular interactions involved in cell adhesion.

In summary, we have demonstrated that phototriggered cleavage of the RGD peptide can lead to subsequent cell release from surfaces. To our knowledge this is the first demonstration of a dynamic variation of RGD concentration at surfaces and its consequences in cell detachment by means of photosensitive molecules.

Experimental Section

The detailed synthesis and characterization of the linker, conditions for surface modification irradiation, and coupling protocols are provided as Supporting Information.

Supporting Information

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

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