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Abstract

Micro-patterning techniques, such as photo-lithography and micro-contact printing, provide robust tools for controlling the adhesive interactions between cells and their extracellular environment. However, the ability to modify these interactions in real time and examine dynamic cellular responses remains a significant challenge. Here we describe a novel strategy to create dynamically adhesive, micro-patterned substrates, which afford precise control of cell adhesion and migration over both space and time. Specific functionalisation of micro-patterned poly(ethylene glycol methacrylate) (POEGMA) brushes with synthetic peptides, containing the integrin binding "RGD" motif, was achieved using thiol-yne coupling reactions. RGD activation of POEGMA brushes promoted fibroblast adhesion, spreading, and migration into previously non-adhesive areas, and migration speed could be tuned by adjusting the surface ligand density. We propose that this technique is a robust strategy for creating dynamically adhesive biomaterial surfaces and a useful assay for studying cell migration.

Keywords: Polymer brush, micro-pattern, click chemistry, cell migration, integrin

1. Introduction

In recent years, the development of micro-patterning techniques in which adhesive or non adhesive proteins and polymers are precisely patterned on cell culture surfaces have provided many important insights into the basic regulatory mechanisms governing cell adhesion and spreading [1,2], as well as their impact on growth [3,4], survival [4], and differentiation [5,6]. Common micro-patterning strategies include micro-contact printing of self-assembled monolayers [4,7,8], UV photo-patterning [9], and direct protein printing [10]. While each of these techniques allows extracellular matrix (ECM) proteins to be deposited in fixed 2D patterns, only a few reports have described systems for creating dynamic or stimuli-responsive patterns [11–13]. For example, light or electric potentials can be used to selectively remove non-adhesive regions from a surface [11,12], while light-activated de-protection [13,14] or host-guest chemistry [15] provide additional means for controlling cell adhesion to various biomaterials.

"Click chemistry" offers yet another approach for creating dynamically adhesive surfaces. Click chemistry refers to a class of reactions, which are highly efficient and specific under physiologic conditions and are attractive for biologic applications. The most well-studied click chemistry reaction is the copper-catalysed azide-alkyne cycloaddition (CuAAC), where an organic azide reacts with an alkyne to form a triazole ring [16]. Until recently, a major disadvantage was the use of toxic Cu (I) as a catalyst [17], but advances in copper-free click chemistry using strained alkynes have since made it possible to perform similar reactions in the presence of cells and create dynamically adhesive substrates [18]. Thiol-ene and thiol-yne reactions belong to another class of click reactions that involve the free-radical mediated addition of thiols to unsaturated carbon-carbon bonds [19,20]. Although both alkene and

alkyne groups can react with a single thiol group, radical additions to alkynes produce vinyl sulfides, which can undergo a second addition of a thiyl radical [19].

Thiol-ene/yne reactions are particularly useful for biological applications since thiol groups are present in cysteines, which can easily be introduced into synthetic peptides or found in some native proteins. However, the biggest advantage of this reaction is its high efficiency and low toxicity, which makes it a good candidate for functionalisation of biomaterials [21]. Light-based activation of thiol-ene/yne reactions also provides the ability to spatially and temporally control functionalisation [22]. In this study, we describe a method to create dynamically adhesive biomaterial surfaces using thiol-ene/yne coupling of cell adhesive ligands to POEGMA brushes. By combining this reaction with micro-contact printing, we developed a tuneable *in vitro* assay, which allows us to control and study cell adhesion and migration over both space and time.

2. Materials and Methods

2.1 Preparation of micro-patterned poly(ethylene glycol) methacrylate (POEGMA) brushes

Micro-patterned polydimethylsiloxane (PDMS, Sylgard 184) stamps were inked with the thiol initiator, ω-mercaptoundecyl bromoisobutyrate, and stamped onto gold-coated coverslips to deposit the initiator as a self-assembled monolayer [6,8]. Non-patterned substrates were soaked in the thiol initiator overnight. Atom transfer radical polymerisation (ATRP) of oligo(ethylene glycol) methacrylate (OEGMA; MW_{avg} 360) was carried out as previously described[6,23]. The polymerisation reaction was performed at room temperature for 1 h, resulting in an estimated 60 nm thick brush[23]. After polymerisation, substrates were activated with N,N'-disuccinimidyl carbonate (DSC, 0.1 M) and dimethylamine pyridine (0.1 M) in N,N-dimethylformamide (DMF), overnight at room temperature. After washing twice

with DMF and water, substrates were incubated overnight at room temperature with 1% allylamine or propargylamine in DMF. Both allylamine and propargylamine were handled inside the fume hood, using protective goggles, gloves and lab coat. All experiments were performed after washing substrates with 70% ethanol for 10 min and twice with phosphate buffered saline (PBS). All reagents and solvents were obtained from Sigma-Aldrich.

2.2 Thiol-ene and thiol-yne reactions

Thiol-ene/yne reactions were performed in the presence of a photoinitiator, Irgacure 2959 (2-Hydroxy-4'- (2-hydroxyethoxy)-2-methylpropiophenone, Sigma-Aldrich) and UV light (~23 mW/cm2, λ_{max} = 365 nm). Irgacure 2959 was resuspended at 0.5% (W/V) in Dulbecco's modified eagle medium (DMEM) without phenol red and allowed to dissolve overnight at 37°C. Substrates were cut to 1 cm^2 and immersed in 500 μl total volume, with varying concentrations of Irgacure 2959 and **C**GG**RGD**SP (RGD) or **C**GG**RGE**SP (RGE) synthetic peptides (Insight Biotechnology Inc., UK). The GG were used as a spacer, and the SP sequence was added to enhance the integrin-RGD binding [24]. The coupling reaction was initiated by exposing the substrates to UV light from an LED array (Cetoni) for specified amounts of time. After exposure, substrates were immediately washed three times with PBS in the absence of cells or DMEM in the presence of cells. For the photo-mask experiments, the reaction was performed in 5 μl total volume of 1 mg/ml RGD and 0.5% Irgacure 2959. The photo-mask was placed on top of the UV lamp, and the substrate was then inverted onto the 5 μl reaction mixture and exposed to 1 minute UV followed by three PBS washes.

2.3 Cell culture, adhesion, and migration

NIH 3T3 fibroblasts and Madin-Darby canine kidney (MDCK) cells were maintained in high-glucose DMEM (Life Technologies) supplemented with 10% or 5% foetal bovine serum (Life

Technologies), respectively, and 1% penicillin/streptomycin (Life Technologies). To examine fluorescence, MDCK cells were transfected with 0.7 μg pEGFP-N1 plasmid DNA (Clontech) and 2.5 μl Lipofectamine 2000 (Life Technoliges) per well of a 6-well plate (70% confluent).

For adhesion assays, substrates were blocked for 1 h with 2% bovine serum albumin in PBS (BSA/PBS), and cells were seeded onto non-patterned substrates in serum-free DMEM at a density of 10,000 cells per cm^2 (1 substrate per well of a 24 well plate) and allowed to adhere for 1h 30min at 37°C, 5% CO_2. Non-adherent cells were rinsed off, and adherent cells were fixed and stained as described. For migration studies, cells were seeded onto alkyne-modified micro-patterned substrates at a density of 50,000 per cm^2, rinsed after 1 h, and cultured overnight. Photo-activated coupling reactions were performed as described above, and migration was monitored by phase contrast imaging with an Incucyte Zoom microscope (Essen Bioscience), image acquisition every 15 min, for 24 h. The Incucyte Zoom image analysis software was used to automatically detect cell edges and generated an overlay mask, which was used to calculate the cell coverage area.

2.4 Enzyme-linked immunosorbent assay (ELISA)

POEGMA substrates were prepared as described and functionalised with the peptide **C**GG**RGD**SP-biotin (RGD-biotin). To ensure the measurements were within the linear range of the ELISA, RGD-biotin was diluted 1:100 with non-biotinlylated RGD. Substrates were blocked for 1 h with 2% BSA/PBS and incubated with 0.1 μg/ml horseradish peroxidase (HRP)-conjugated streptavidin (Thermo Fisher) for 1h at room temperature. After three washes with PBS, substrates were incubated with the one-step 3,3',5,5'-tetramethylbenzidine (TMB) ELISA buffer (Thermo Fisher), to detect HRP activity, for 15 min at room temperature. The reaction was stopped with 2 M sulfuric acid. Absorbance was measured at

450 nm in a micro-plate spectrophotometer (BMG Labtech).

2.5 Immunofluorescence staining and imaging

Cells were fixed with 4% paraformaldehyde for 10 min at room temperature and permeabilised with 0.2% TritonX-100/PBS for 10 min. Samples were blocked for 1 h with 2% BSA/PBS and antibodies were diluted in a solution of 2% BSA/PBS and incubated for 1 h at room temperature. Coverslips were mounted on glass slides with Mowiol reagent (Sigma-Aldrich), and images were acquired with a DM5000B epi-fluorescence microscope (Leica) or LSM 710 confocal microscope (Carl Zeiss Inc.). The mouse anti-paxillin antibody (P13520; 1:500; BD Biosciences), DAPI (1:1000) and Phalloidin Alexafluor 488 (1:1000; Invitrogen) were used.

2.6 Statistical Analyses

For quantification of adherent cells, the numbers of DAPI positive nuclei were counted in 5 random images per sample. Triplicates for each condition were analysed for three individual experiments with image J software. Migration was quantified with the Incucyte Zoom image analysis software or ImageJ by measuring the cell coverage of at least 4 micro-patterned islands per condition in 3 independent experiments and reported as the percentage change in cell area. All data were reported as the mean ± standard error of the 3 experiments and analysed by two-tailed unpaired T-Tests. $P < 0.05$ determined significance.

3. Results

3.1 Characterisation of thiol-ene/yne reaction efficiency

The overall synthesis and reaction strategy is described in Figure 1. First, POEGMA brush substrates were generated by surface initiated polymerisation as previously described [6,23].

Hydroxyl-terminated POEGMA brushes were then activated with disuccinimidyl carbonate (DSC) [23], and alkene or alkyne modifications were performed by incubating the activated brushes with allylamine or propargylamine, respectively (Fig. 1A). To confer cell adhesive properties, modified POEGMA brushes were functionalised with a synthetic peptide (**C**GG**RGD**SP-biotin) containing the arginine-glycine-aspartic acid (RGD) motif, which is present in many extracellular proteins, such as fibronectin and vitronectin, and recognised by integrin receptors [25]. Thiol "click" reactions between the N-terminal cysteine of the peptide and the alkene or alkyne modified brushes were initiated by long wave UV (23 $mWcm^{-2}$, λ_{max} = 365 nm) and the photo-initiator Irgacure 2959 (Fig. 1B). For migration studies, NIH 3T3 mouse fibroblasts or MDCK epithelial cells were seeded onto micro-patterned islands surrounded by alkyne modified POEGMA brushes, and cell migration over the polymer surface was induced by thiol-yne functionalisation with the RGD peptide (Fig. 1C).

A modified enzyme-linked immunosorbance assay (ELISA) was used to characterise the efficiency of the thiol-ene/yne reactions and dependence on several key parameters. Quantification of the relative amount of biotin-tagged peptide on the polymer brush surface indicated that the reaction requires both UV and PI (Fig. 2A). Consistent with previous findings [22,26], reaction efficiency increased in a dose-dependent manner with increasing UV exposure and PI concentration before saturating at 5 minutes UV and 0.5% PI. Although the reaction with 5 min UV (0.5% PI) was twice as efficient as 1 min UV, this condition was toxic to cells (Supplementary Fig. S1). Therefore, 0.5% PI and 1 min UV exposure were used in all subsequent experiments as this condition provided a good combination of efficiency and cell viability. We also find that 1 min UV exposure does not adversely affect GFP fluorescence in living cells (Supplementary Fig. S2).

For both the alkene and alkyne brushes, RGD surface density increased with increasing input RGD concentrations and reached a maximum between 0.8 and 1 mg/ml (Fig. 2B). At low RGD concentrations, the resulting peptide surface density was up to 100% higher for the alkyne-modified brushes, but when the reaction saturated at 1 mg/ml RGD, the reaction efficiency was similar for both alkene and alkyne surfaces. These results indicate that both thiol-ene and thiol-yne reactions could be used for functionalisation of POEGMA brushes but that the thiol-yne was generally more efficient. The higher overall efficiency on the alkyne surfaces is likely due to the ability of alkyne groups to react doubly with thiols [19]. The final peptide surface density could also be controlled by varying the ratio of OH versus CH_3 terminated POEGMA and thus the availability of DSC reactive groups (Fig. 2C). Finally, we prove that the reaction depends specifically on the cysteine residue by competing out the peptide coupling with free cysteine. The addition of 10X and 50X molar excess of cysteine, but not glycine, to the reaction significantly reduced RGD peptide density in a dose-dependent manner (Fig 2D).

3.2 Bio-activity of RGD functionalised polymer brushes

We next determined whether RGD peptides coupled to POEGMA brushes via the thiol-ene and thiol-yne reactions were biologically active by examining fibroblast adhesion and spreading. Cells were seeded onto non-patterned POEGMA brushes treated with RGD only, UV and PI, RGD plus UV and PI, or the non-functional peptide (RGE) plus UV and PI. Cells adhered specifically to covalently bound RGD in comparison to RGD only treatment, UV plus PI treatment, or RGE modified surfaces (Fig. 3A). Furthermore, fibroblasts on the RGD functionalised surfaces were more spread and formed actin stress fibres and focal adhesions (Fig. 3B, E). Cell adhesion increased with increasing RGD surface density on POEGMA brushes reacted with 0, 0.3, 0.5 and 1 mg/ml RGD (Fig. 3C). The step change in adhesion from 0.5 mg/ml to 1 mg/ml may be due to crossing the threshold in surface ligand density

required for integrin clustering [27]. Taken together, these results demonstrate that RGD peptides coupled to POEGMA brushes are biologically functional and promote cell adhesion and spreading.

We also compared cell adhesion on thiol-ene and thiol-yne modified brushes reacted with 1 mg/ml RGD, 0.5% PI, and 1 min UV. Although the ligand density was similar for these two conditions, cell adhesion was significantly higher (two-fold) on the alkyne-modified brushes compared to alkene (Fig. 3D). However, the cells that adhered to both surfaces appeared to spread and form focal adhesions and stress fibres (Fig. 3E). These results indicate that despite similar surface ligand densities, the alkyne modified surfaces promoted greater cell adhesion and were therefore used in all subsequent studies.

3.3 RGD functionalisation of micro-patterned POEGMA induces cell migration

To determine whether thiol-yne chemistry could be used to create dynamically adhesive surfaces and promote cell migration, we first examined RGD functionalisation of micro-patterned POEGMA brushes. Micro-contact printing and surface initiated polymerisation were used to create patterned substrates with 400 μm diameter gold islands surrounded by POEGMA brushes [18]. As before, substrates were alkyne modified and reacted with 0.5% PI and 1 min UV over a range of input RGD concentrations. Detection of biotin-tagged RGD with FITC-streptavidin revealed that peptide surface density increased with increasing input peptide concentration (Fig. 4A), consistent with the ELISA measurements (Fig. 2B). UV exposure through a photo-mask was also used to create micro-patterned lines (20 μm width) of the RGD peptide on the polymer brushes and thus, a second level of micro-patterned adhesive cues (Fig 4A). These results confirmed that the thiol-yne reaction was compatible with micro-patterned POEGMA and could be used to preferentially functionalise the polymer brush areas.

We then investigated cell migration over the polymer brush surfaces upon activation with the RGD peptide. Following alkyne modification of the POEGMA brushes, fibroblasts were seeded onto the micro-patterned substrates and only adhered to the bare gold islands (Fig. 4B). Cells were cultured overnight before reaction with 0, 0.1, 0.3, 0.5 and 1 mg/ml of RGD or 1 mg/ml RGE (Fig. 4B,C). Live-cell imaging over a 24 h period demonstrated that fibroblasts specifically migrated onto the RGD functionalised brushes and not on the RGE brushes (Fig. 4B, Supplementary Movie 1,2). We confirmed that this response was due to migration and not just proliferation by inducing migration in cells treated with Mitomycin C, which permanently blocks proliferation (Supplementary Movie 3). The influence of RGD surface density on cell migration was quantified by measuring the percentage change in cell coverage area over time (Fig. 4C). We found that coverage area increased with increasing ligand density (Fig. 4C), and cell coverage 4 hours after functionalisation was significantly higher on the surfaces treated with 0.3, 0.5 and 1 mg/ml RGD compared to the RGE control (Fig. 4D). F-actin stress fibres and paxillin containing focal adhesions could be observed in cells on the RGD functionalised brushes (Fig. 4E), further demonstrating that migration onto these surfaces was an integrin-mediated process and similar in nature to migration on native ECM proteins. We conclude that thiol-yne functionalisation of POEGMA brushes with RGD peptides can be used to induce cell migration and that migration speed can be tuned by altering surface ligand density.

To further investigate the flexibility of this model system, we examined how migration depended on the timing of introduction of the new ligand and compatibility with other cell types. RGD peptides were coupled to alkyne-modifed brushes either 5 hours or 27 hours after cell seeding, and no differences in the ability of fibroblasts to migrate onto the brushes were observed at 4 hours and 24 hours after activation (Fig 5A). In addition, Madin-Darby canine

kidney (MDCK) cells rapidly migrated onto RGD-functionalised brushes following thiol-yne coupling (Fig 5B). These results indicate that the timing of RGD functionalisation within a 22 hour window does not substantially affect migration and that this model system is compatible with multiple cell types.

4. Discussion

In this study, we employed thiol-yne chemistry and micro-contact printing to create a tuneable cell migration assay. While patterned POEGMA brushes specified the orientation and geometry of the migration area, the thiol-yne reaction determined the composition and ligand density on the surface. To demonstrate a potential application for this model system, we show that the surface density of the RGD ligand can be used to control the rate of cell migration. Furthermore, light-based activation allows adhesive ligands to be introduced at various times and locations, and the overall approach is compatible with both fibroblast and epithelial cell types. This experimental system is therefore highly flexible and capable of examining how different types of extracellular signals impact dynamic cellular processes.

While the thiol-yne reaction described here allows specific ligands to be added onto a protein resistant background, our findings identified a few notable limitations for biological applications. Longer UV exposure and higher PI concentrations generate free radicals, which are toxic to cells, and limit the amount of coupling that can be achieved while maintaining cell viability. Azide-alkyne cycloaddition provides an alternative click chemistry approach that avoids the use of UV and PI [18]. Although the reagents for these reactions are more difficult to synthesise, this strategy may be preferred when cell viability is an issue. Another point to consider is that thiol-ene/yne reactions require free thiols, which are easy to introduce as cysteines in a synthetic peptide, but may not always be available in native proteins with

disulfide bonds. Finally, we find significantly better cell adhesion on alkyne surfaces compared to alkene surfaces, despite only small differences in peptide density. We hypothesise that the alkene modification interferes with integrin binding to the RGD modified brushes, and this effect will be investigated further in future studies.

The techniques developed in this study have a broad range of potential biological applications. As a research tool, these assays could be used to study fundamental aspects of cell migration, which is essential for wound healing, neurite extension, and angiogenesis. Scaling-up this platform would also make it amenable to high-throughput screening of drugs or therapeutics [28]. As each substrate contains hundreds of identical 'micro-wounds', the quantity and consistency of data that can be collected from a single experiment would be significantly improved compared to traditional scratch assays used for migration studies. Beyond *in vitro* assays, thiol-yne functionalisation of polymer brush coatings could be advantageous for creating dynamically adhesive or stimuli responsive biomaterials for medical devices or bio-sensing applications [29,30].

5. Conclusions

In conclusion, we demonstrate that thiol-yne reactions can be used to introduce adhesive ligands in the presence of cells. This method is specific, fast (carried out in only one minute), and compatible with cell culture conditions. When combined with micro-patterned polymer brushes, thiol-yne functionalisation can be used to create dynamically adhesive cell substrates and tuneable migration assays. Therefore, it is a robust technique with a wide range of potential applications. We propose that it could be a powerful tool for studying the influences of the cellular microenvironment on migration processes.

Acknowledgements

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Figures

Figure 1

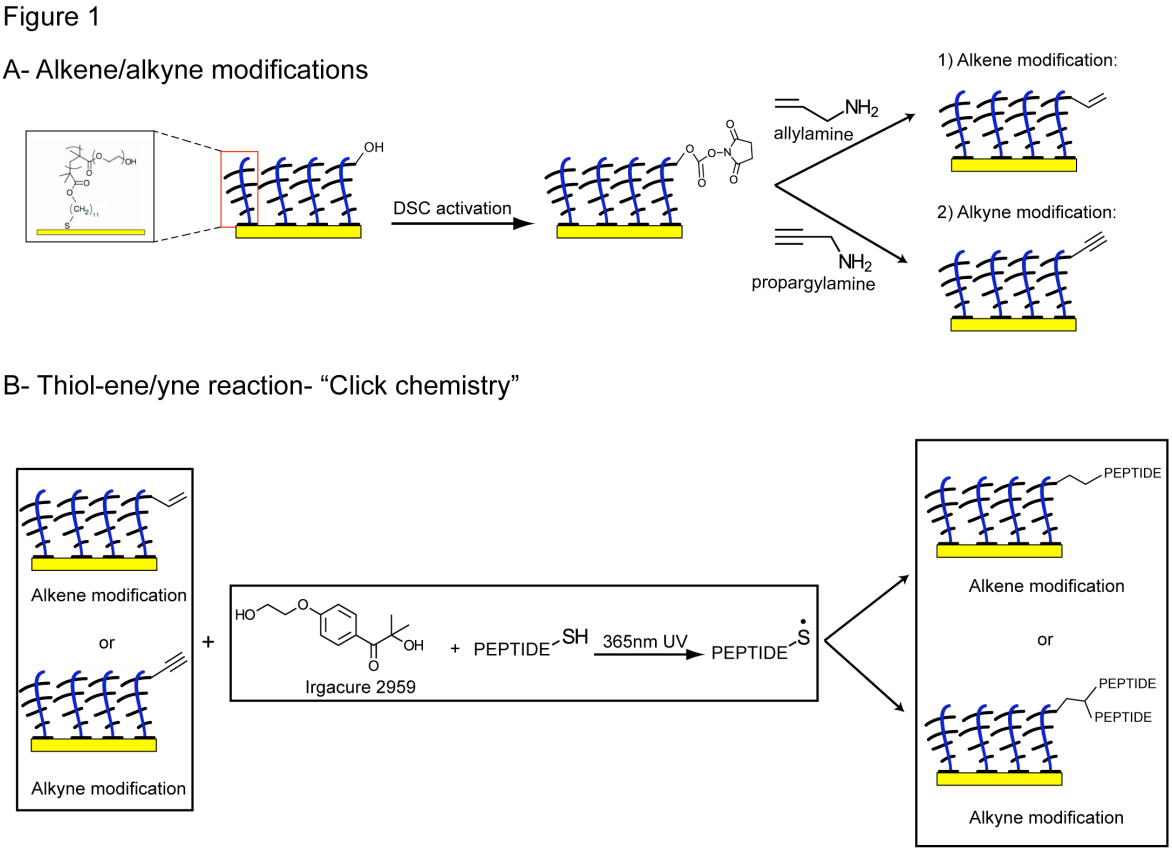


Fig. 1. Schematic for thiol-ene/yne functionalisation of POEGMA brushes. (A) POEGMA brushes were synthesised by surface initiated polymerisation, followed by activation of the terminal hydroxyl groups with DSC. Activated brushes were then reacted with allylamine or propargylamine to generate alkene or alkyne modifications, respectively. (B) Alkene and alkyne modified POEGMA brushes were reacted with a thiol radical, which was generated by exposure to long wave UV light and a PI (Irgacure 2959). Reaction products are peptide functionalised POEGMA brushes. (C) To create an inducible cell migration assay, micro-patterned POEGMA brushes were first created by micro-contact printing. Following alkyne

modification, cells were plated onto the patterned substrates, and thiol-yne functionalisation with RGD peptides promoted cell migration over the polymer brushes.

Figure 2

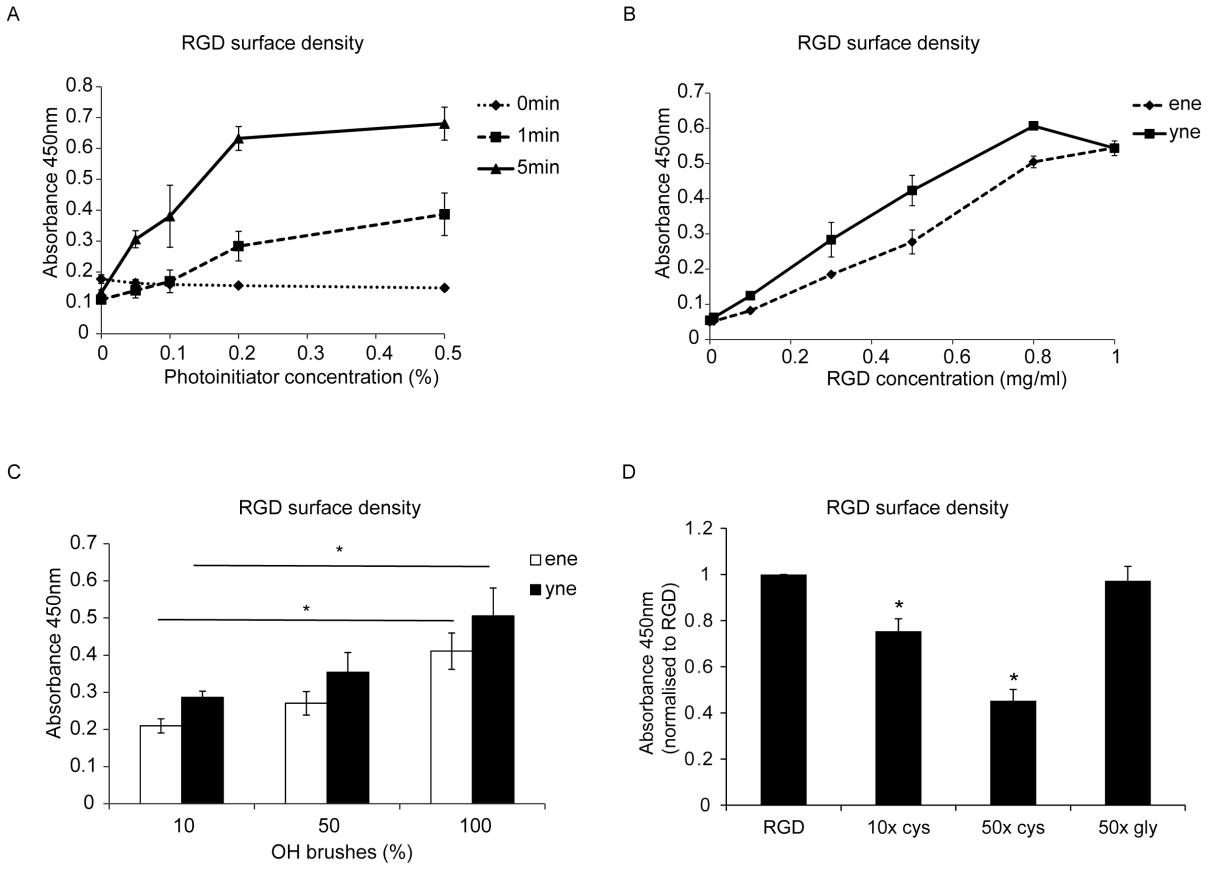


Fig. 2. Characterisation of thiol-ene/yne reaction conditions. Non-patterned POEGMA surfaces were functionalised with a biotinylated RGD peptide, and surface ligand density was measured by ELISA. (A) The effect of UV exposure and PI concentration was determined by reacting alkyne-modified POEGMA with 0.5 mg/ml RGD and treatment with 0, 1, or 5 min UV (23 mW cm^{-2}) and 0, 0.05, 0.1, 0.2, 0.5% PI (Irgacure 2959). (B) The dependence of RGD surface density on input peptide concentration was examined by reacting alkene and alkyne modified surfaces with 0, 0.1, 0.3, 0.5, 0.8, and 1 mg/ml RGD (1 min UV, 0.5% PI). (C) The influence of POEGMA chemistry on surface ligand density was examined by creating POEGMA brushes with mixed ratios of –OH to $–CH_3$ terminated side chains, consisting of 10, 50, or 100% OH. Polymer brushes were then functionalised with 0.5 mg/ml RGD, 1 min UV, and 0.5% PI. (D) Reaction specificity was examined by measuring RGD surface density when

reacted with 10X (6.7 mM) or 50X (33.5 mM) molar excess of cysteine or 50X (33.5 mM) excess glycine. Measurements were normalised to the RGD only control levels. All other data are expressed as ELISA absorbance measurements at 450 nm and represent the mean ± SEM of 3 independent experiments (*P < 0.05).

Figure 3

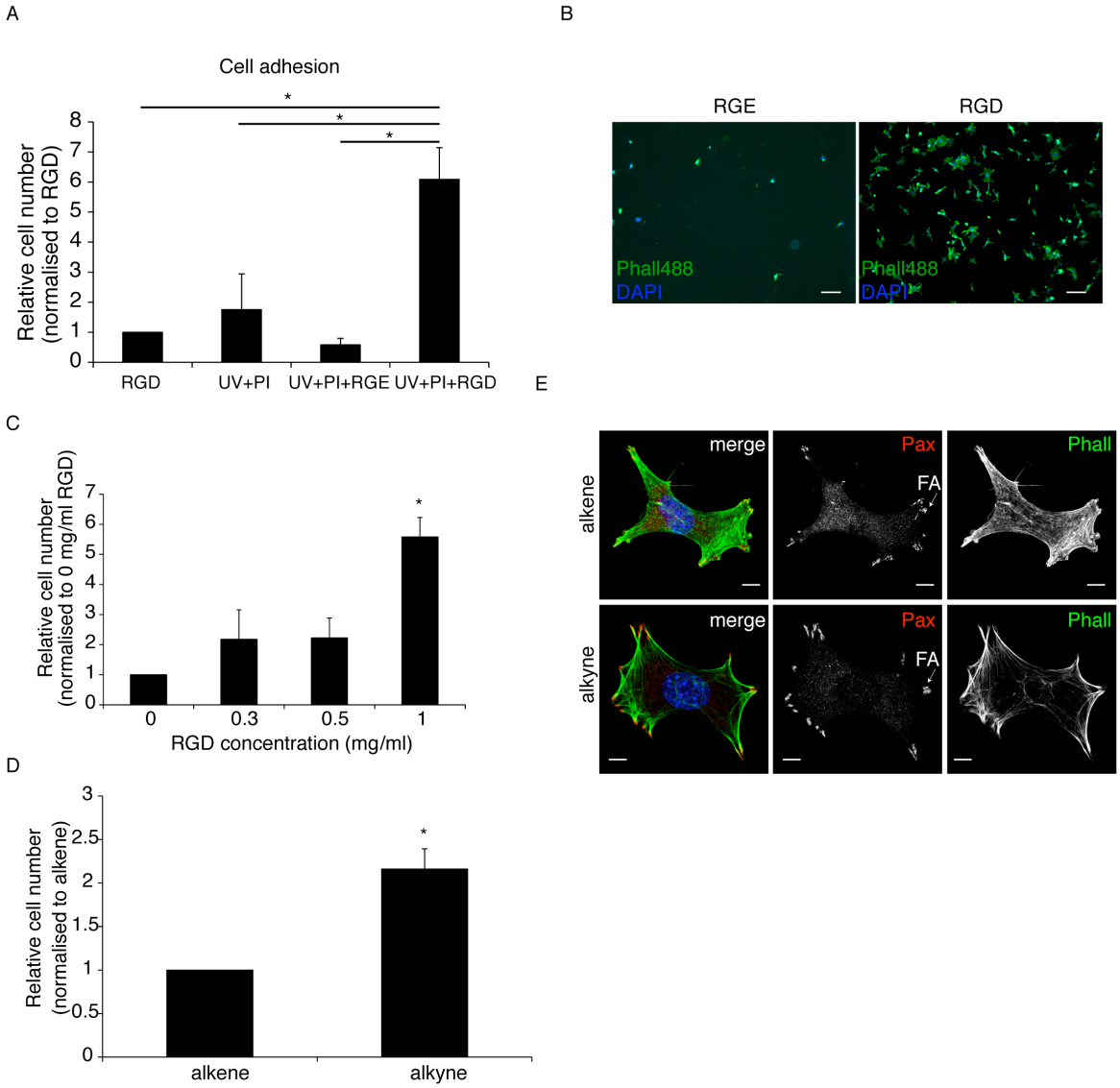


Fig. 3. Influence of RGD functionalisation on fibroblast adhesion and spreading. (A) Fibroblast adhesion to RGD functionalised brushes was measured following treatment of non-patterned, alkyne-modified POEGMA surfaces with 1 mg/ml RGD, 1 min UV plus 0.5% PI, 1 mg/ml RGD plus UV and PI, or 1 mg/ml RGE plus UV and PI. (B) Representative images show F-actin staining (phalloidin) of cells on RGE and RGD functionalised surfaces. Scale

bar is 100 μm. (C) The effect of surface ligand density was examined by measuring cell adhesion to substrates functionalised with 0, 0.3, 0.5, and 1 mg/ml RGD (1 min UV, 0.5% PI). (D) Cell adhesion was measured and compared for alkene and alkyne brushes reacted with 1 mg/ml RGD, 1 min UV, and 0.5% PI. In all experiments adhesion was quantified by counting the number of cells in 15 random fields per condition. Data are expressed as cell number relative to an internal control and represent the mean ± SEM of 3 independent experiments (*$P < 0.05$). (E) Representative images show focal adhesion (paxillin) and F-actin localisation (phalloidin) on alkene and alkyne surfaces functionalised with RGD peptides. Scale bars equal 10 μm.

Figure 4

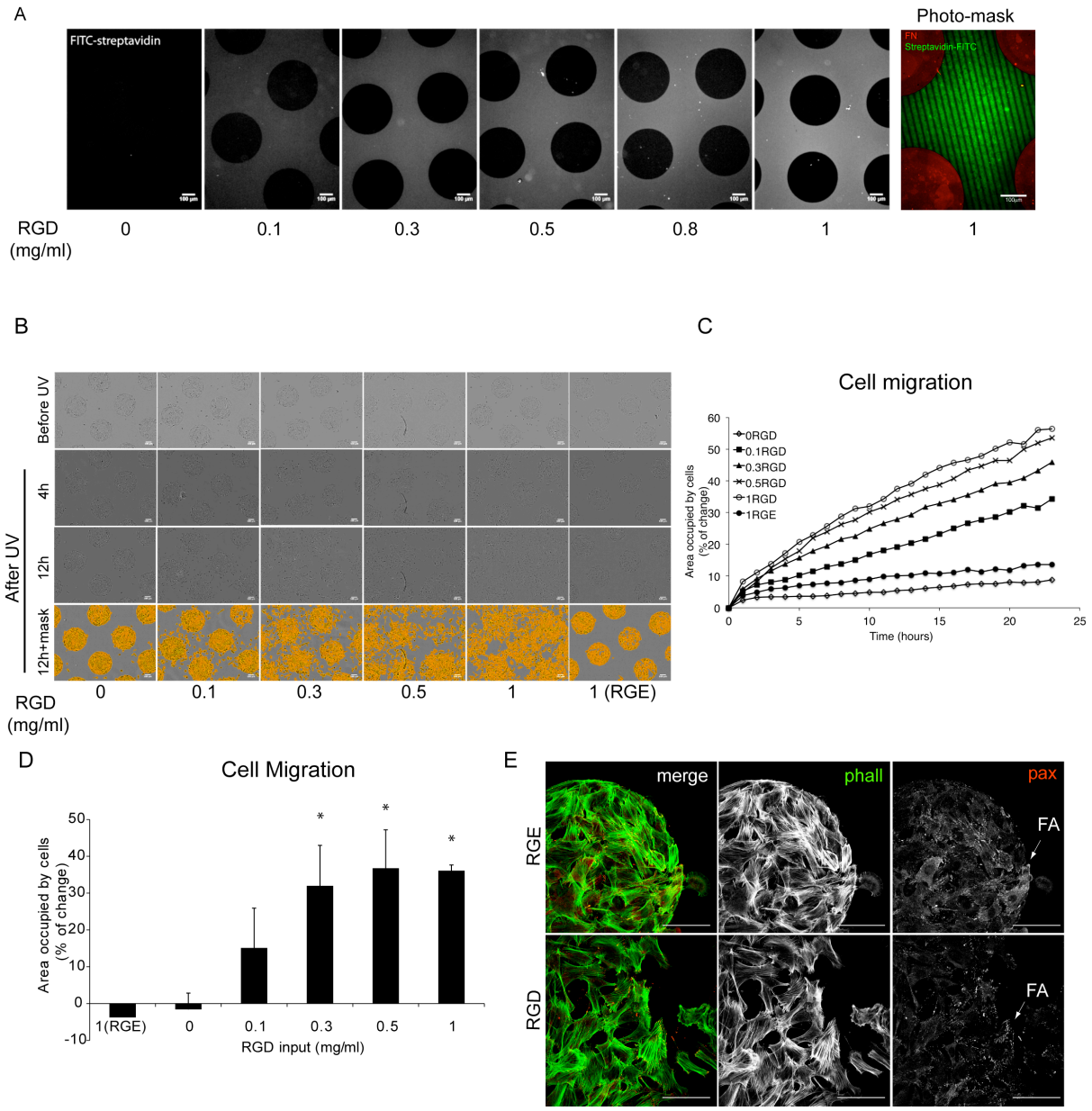


Fig. 4. Activation of cell migration on micro-patterned polymer brushes. (A) Micro-patterned POEGMA brushes surrounding 400 μm gold islands were alkyne modified and functionalised with RGD-biotin (0 to 1 mg/ml). The end panel shows functionalisation of POEGMA brushes with 1mg/ml RGD, surrounding fibronectin-coated 400 μm islands (red), when exposed to UV through a photo-mask with 20 μm lined openings. RGD peptides were detected with FITC-streptavidin. (B) Cell migration on the micro-patterned substrates was activated by reaction of thiol-yne modified brushes with 0, 0.1, 0.3, 0.5, or 1 mg/ml RGD or 1 mg/ml RGE. Representative phase contrast images show cell migration at 0, 4, and 12 h after

reaction and overlayed with the cell mask (yellow) generated by the Incucyte Zoom software. (C) Cell migration was quantified by measuring the coverage area using the cell mask. Plots show the percentage change in cell coverage area of representative images for 0, 0.1, 0.3, 0.5, and 1 mg/ml RGD functionalisation over 24 h. (D) The effect of RGD surface density on cell migration was quantified by the percentage change in cell coverage area 4 h after activation. Data represent mean ± SEM of 3 independent experiments (*P < 0.05 compared to RGE). (E) Representative images of focal adhesion (paxillin) and F-actin (phalloidin) localisation on RGE and RGD functionalised patterns. Arrows indicate focal adhesions (FA). All scale bars are 100 μm.

Figure 5

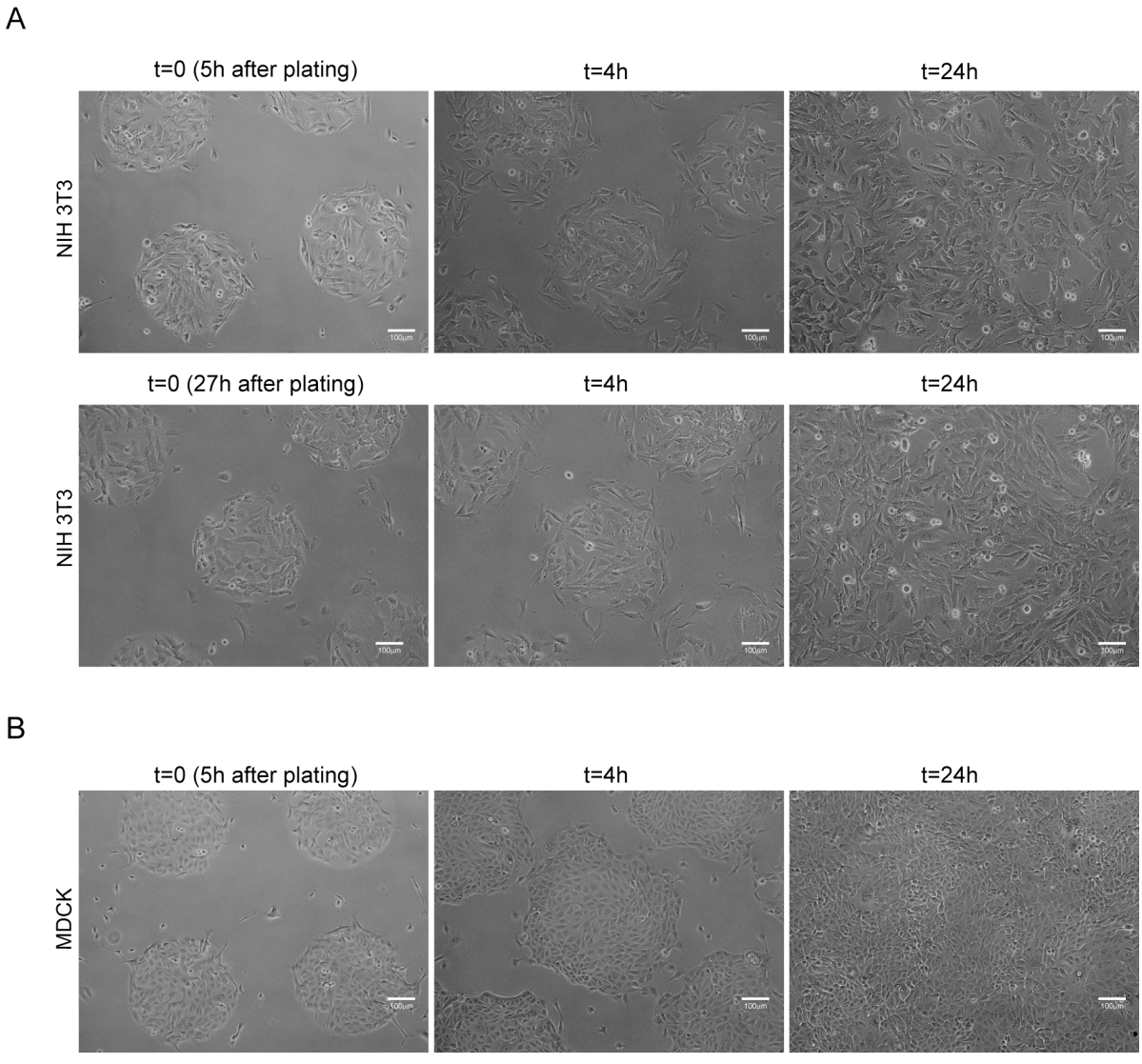
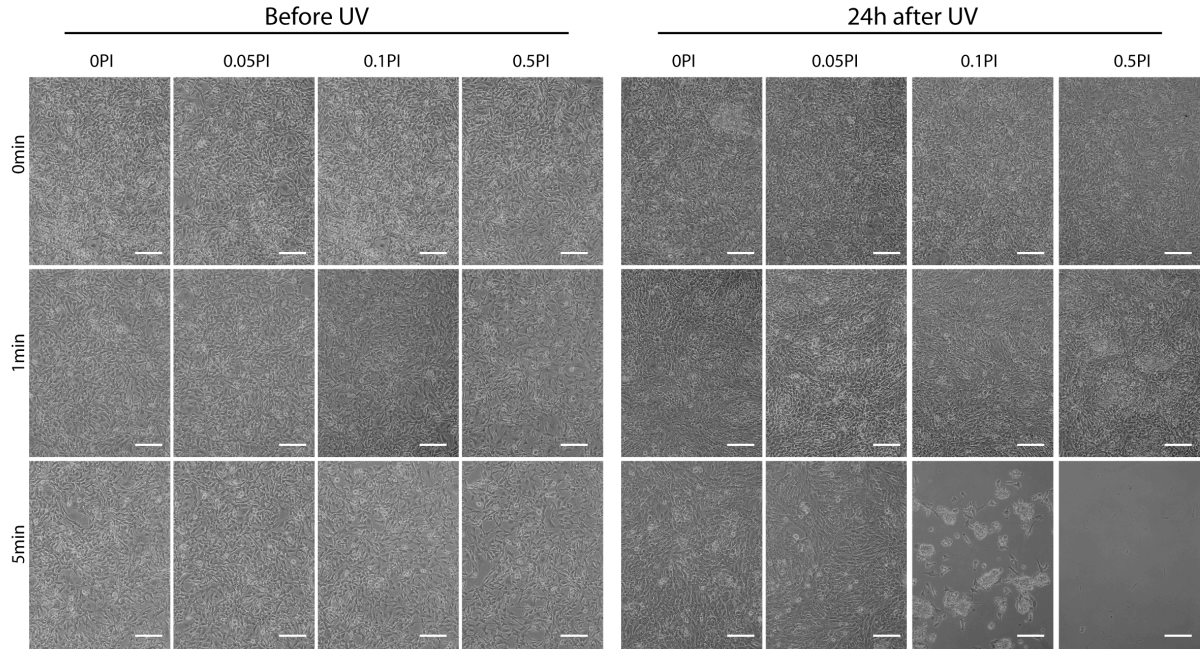


Fig. 5. Influences of reaction timing and cell type on migration. (A) NIH 3T3 fibroblasts were plated onto 400 μm islands. Alkyne-modified POEGMA brushes were functionalised with 1 mg/ml RGD 5h and 27h after plating, and migration was observed 4h and 24h later by phase-contrast imaging. (B) MDCK cells were plated onto 400μm islands. POEGMA brushes were functionalised with 1 mg/ml RGD 5h after plating and migration was observed 4h and 24h later.

Supplementary Information

Figure S1

A



B

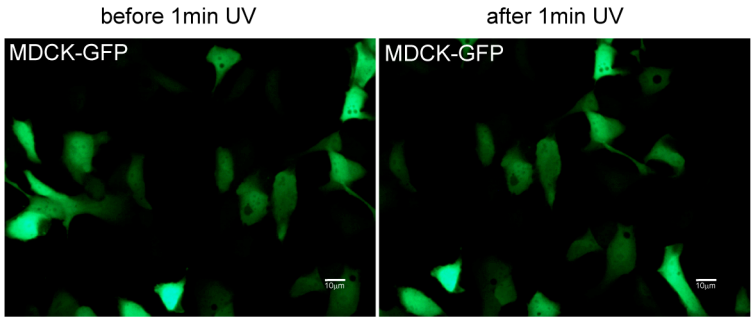


Fig. S1. Effect of UV and photo-initiator on cell viability and fluorescence. (A) NIH 3T3 fibroblasts were cultured in 24-well tissue-culture plates and treated with 0, 0.05, 0.1, or 0.5% PI and 0, 1, or 5 min UV. Representative images before and 24 h after treatment demonstrate identical phenotype in all conditions, except those treated with 5 min UV with 0.1% and 0.5% PI. (B) GFP- expressing MDCK cells were treated with 1 min UV. Representative images show no differences in GFP levels before and after UV treatment.