Supplementary Material for

Spacing of integrin ligands influences signal transduction in endothelial cells

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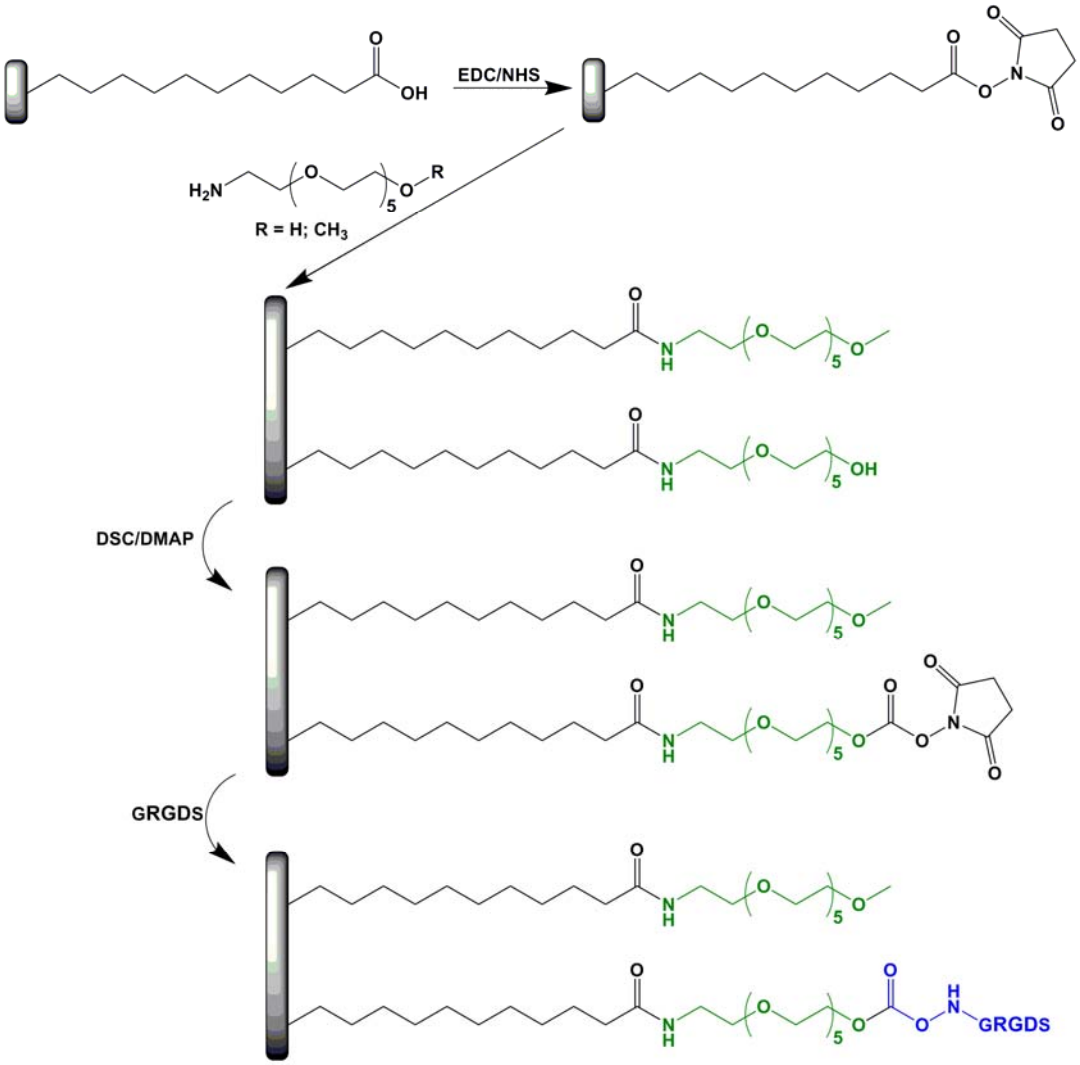
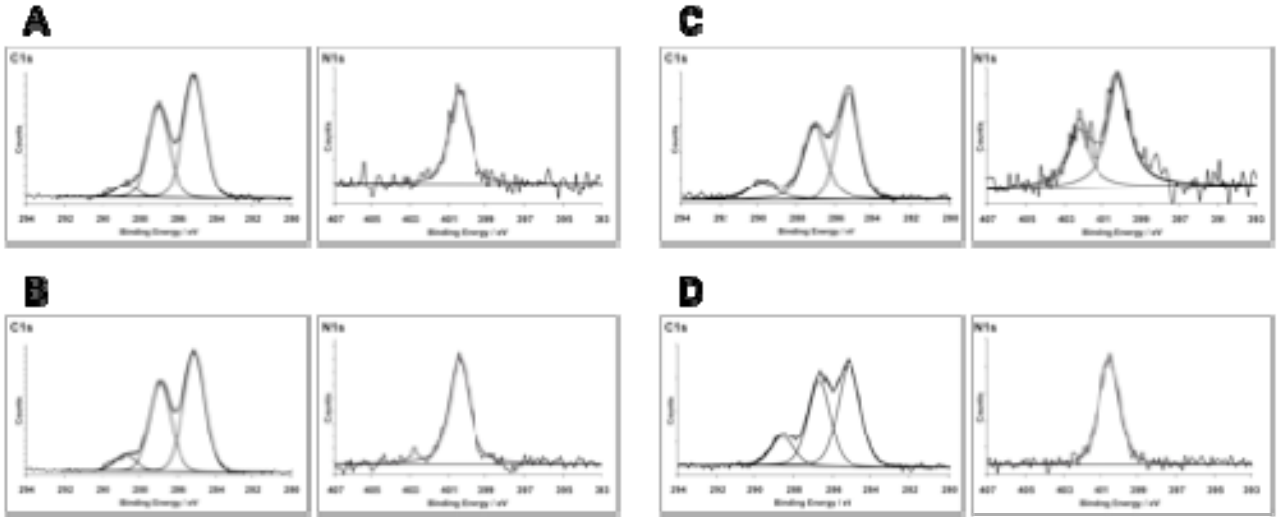


Supplementary Figure 1: Reaction scheme illustrating the different steps involved in the modification of silicon surfaces for cell adhesion studies as detailed in the methods section.



Supplementary Figure 2: X-ray photoelectron narrow scans of the C1s and N1s regions of an undecanoic acid monolayer on Si(100) after activation with EDC/NHS followed by coupling of **(A)** 1-amino hexa(ethylene oxide) monomethyl ether or **(B)** 1-amino hexa(ethylene oxide). **(C, D)** Successive derivatisation steps on the hydroxyl-terminated surface shown in panel B: **(C)** activation with DSC/DMAP and **(D)** coupling of the GRGDS peptide.

Estimation of coupling yields:

In the first coupling reaction the undecanoic acid monolayer was activated with EDC/NHS followed by reaction with 1-amino hexa(ethylene oxide) monomethyl ether (EO_6-OMe) or 1-amino hexa(ethylene oxide) (EO_6-OH) or mixtures of these compounds. The nitrogen 1s narrow scans of surfaces modified with either EO_6-OMe (Figure S2A) or EO_6-OH (Figure S2B) showed a single peak at 400.3 eV, characteristic of the amide nitrogen arising from the reaction of these compounds with the activated NHS esters on the surface. The absence of a peak at higher binding energies revealed that no NHS ester groups remained on the surface as the NHS nitrogen would appear at 402.3 eV. Thus, all NHS ester groups were removed during the coupling reaction either via aminolysis or hydrolysis. The carbon 1s envelopes of the surfaces modified with EO_6-OMe (Figure S2A) or EO_6-OH (Figure S2B) were deconvoluted into three peaks. Two peaks were assigned to the base monolayer: The large peak at 285.0 eV corresponds to the C-C bonded carbons of the alkyl chains while the small peak at 288.6 eV arises from the C=O carbons of the amide and unreacted carboxylic acid groups. The peak at intermediate binding energy (286.8 eV) was assigned to the carbons of the hexa(ethylene oxide) moiety introduced during the coupling reaction. The yield of the coupling step estimated on the basis of the peak area ratio was between 40 – 50 % for both hexa(ethylene oxide) species (see Table S1 and Table S2). In this calculation the peak areas were corrected for attenuation of photoelectrons arising from different layers (1).

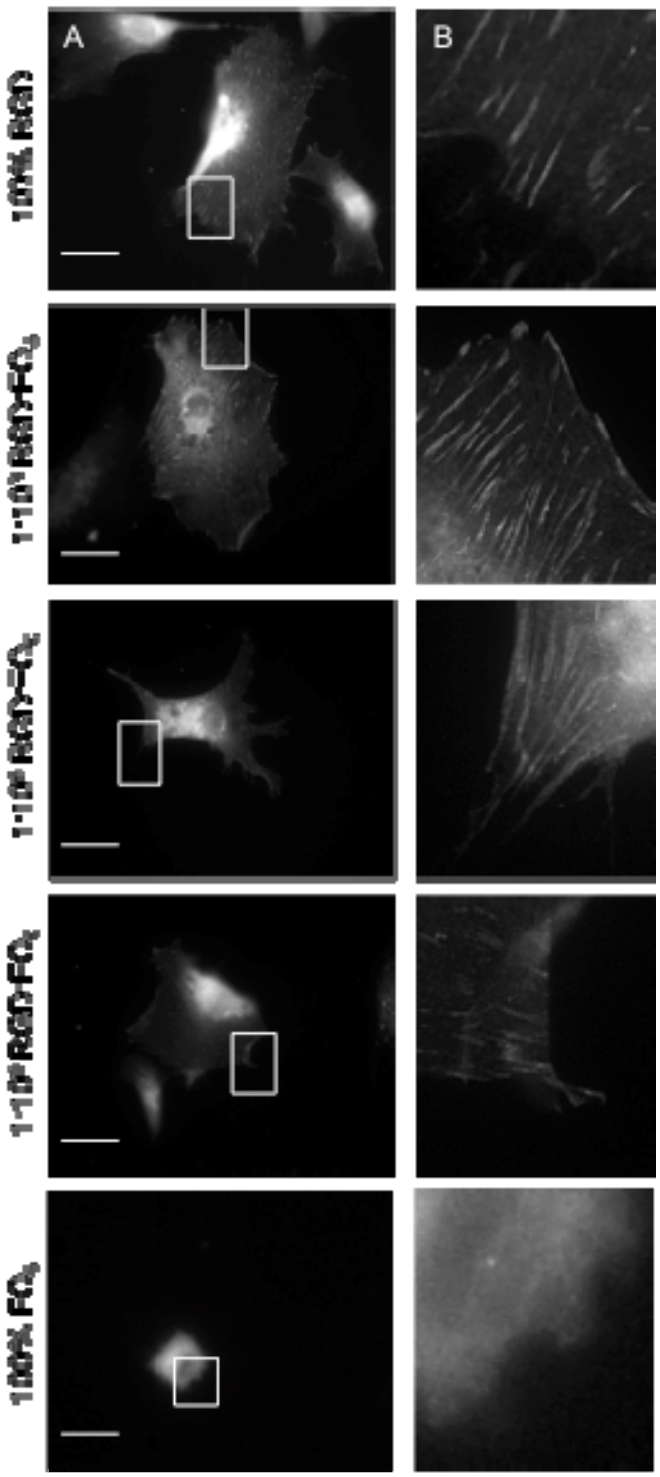
The yield of RGD coupling was determined on a surface modified with EO_6-OH. To activate the terminal hydroxyl groups, the surface was reacted with DSC/DMAP. The nitrogen 1s narrow scan showed that about half of the hydroxyl groups were activated with the appearance of a peak at 402.7 eV arising from the succinimide nitrogen (Figure S2C). Importantly, this peak disappeared after reaction of the activated surface with the RGD peptide indicating that no NHS groups remained on the surface (Figure S2D). The carbon 1s narrow scan of the RGD modified surface showed an increase in the peaks at higher binding energies relative to that at 285.0 eV (Figure S2D) as expected for the introduction of the peptide. On the basis of the peak area ratios (3.81 : 2.96 : 1.00 for the peaks at ~285 eV, 287 eV and 289 eV in Figure S2D) we estimated that approximately 30 – 40% of the EO_6-OH molecules were modified with an RGD peptide, consistent with previous studies utilising the same coupling chemistry (38% (2) and 35% (3). Thus, the *overall* yield of RGD coupling was ~10 – 20% (relative to the chains in the undecanoic acid monolayer).

Supplementary Table S1. Number of carbons of each layer assigned to the peaks at low, intermediate and high binding energy in the carbon 1s narrow scan.

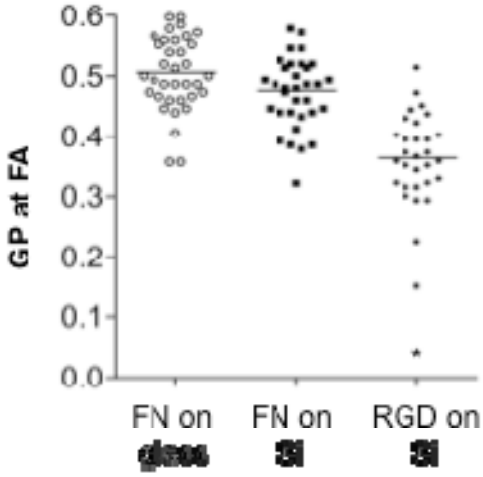
Species	~285 eV	~287 eV	~289 eV
undecanoic acid	10		1
EO_6-OMe		13	
EO_6-OH		12	
GRGDS	3	7	8

Supplementary Table S2. Area ratio of the peaks at low, intermediate and high binding energy in the carbon 1s narrow scan for surfaces A and B. Peak areas were corrected for attenuation of photoelectrons within the layers.

Surface	~285 eV	~287 eV	~289 eV
A. undecanoic acid + EO_6OMe	10	5.82	0.97
	10	6.18	0.98
B. undecanoic acid + EO_6OH	10	5.79	1.13
	10	5.5	0.84



Supplementary Figure 3: Focal adhesions on functionalized silicon surfaces. (**A**) Serum-starved endothelial cells expressing paxillin-GFP were re-plated onto silicon surfaces with various ratios of RGD to EO_6 (as indicated) for 3 h in serum-containing media, fixed and imaged with an epi-fluoresence microscope. (**B**) Magnified regions of focal adhesions at the cell edges as indicated in A. Bars = 20 µm.



Supplementary Figure 4: Membrane order at focal adhesions. Serum-starved endothelial cells were replated onto glass surfaces coated with fibronectin (FN), silicon surfaces (Si) coated with fibronectin or silicon modified with 100% RGD. GP values of paxillin-positive pixels were determined as described for Figure 4. Each symbol represents the mean GP value of one image; means of means are indicated by horizontal bars. The GP value at focal adhesions in cells on 100% RGD surfaces is significantly lower ($P < 0.01$) compared to either of the two fibronectin-coated surfaces. No difference in focal adhesion membrane order between cells on fibronectin-coated glass and silicon was found showing that the surface modification but not the substratum material, influences focal adhesion structure.

References

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