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Electrically-driven modulation of surface-grafted RGD peptides for manipulation of cell adhesion

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Supporting Information

Material and Methods

Chemicals and Materials

Commercially available chemicals and solvents were purchased from Aldrich Chemicals and Fisher Chemicals and were used as received. The oligopeptides C3K-GRGDS and C6EG-GRGDS (Figure S1) were synthetised by Peptide Protein Research Ltd. (Wickham, UK) to > 95% purity and verified by HPLC and mass spectrometry. The (11-mercaptoundecyl)tri(ethylene glycol) (C11TEG), Fetal Bovine Serum (FBS) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (1 M) were purchased from Sigma Aldrich and used as received. Neutravidin and DMEM were obtained from Invitrogen Life Technologies. Phosphate buffered saline (PBS) solution was prepared from a 10× concentrate PBS solution (1.37 M sodium chloride, 0.027 M potassium chloride, and 0.119 M phosphate buffer) from Fisher BioReagents. Polycrystalline gold substrates were purchased from George Albert PVD, Germany and consisted of a 50 nm gold layer deposited onto glass covered with a thin layer of chromium as the adhesion layer.

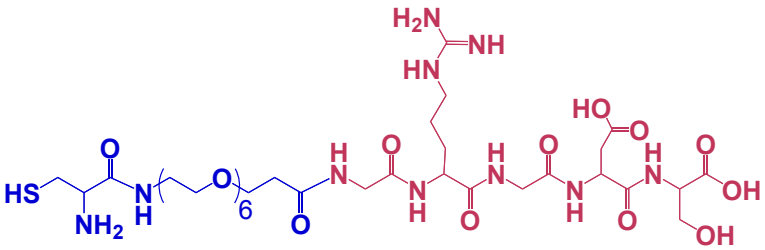

Figure S1 - Chemical structure of the C6EG-GRGDS oligopeptide.

Preparation of Mixed Self-Assembled Monolayers (SAMs)

The Au substrates were cleaned by immersion in piranha solution (3:1, H_2SO_4 : 30% H_2O_2) at room temperature for 10 min, rinsing with Ultra High Pure (UHP) H_2O and then HPLC grade

EtOH thoroughly for 1 min. (*Caution: Piranha solution reacts violently with all organic compounds and should be handled with care*). For the preparation of the C3K-GRGDS:C11TEG or C6EG-GRGDS:C11TEG mixed SAMs, solutions of the oligopeptide (0.1 mM) and C11TEG (0.1 mM) were prepared in HPLC EtOH containing 3% (v/v) $N(CH_2CH_3)_3$, and mixed at the volume ratio of 1:40. Subsequently, the clean Au substrates were immersed in the mixed solutions for 24 h to form the mixed SAMs on the Au surfaces. The substrates were rinsed with HPLC EtOH, an ethanolic solution containing 10% (v/v) CH_3COOH, and UHP H_2O. Note that the mixed SAMs were deposited in the presence of $N(CH_2CH_3)_3$ to prevent the formation of hydrogen bonds between the NH_2 functional groups of the bound thiolate peptide on the Au surface and that of free thiol peptide in the bulk solution.[1] The pure C11TEG SAMs were prepared by immersing the clean gold substrates in ethanolic 0.1 mM solution of the ethylene glycol thiol for 24 h, followed by rinsing with HPLC EtOH.

Cell culture

RAW 264.7 macrophage cells (ATCC# TIB-71) were cultured in supplemented DMEM (10% FBS, 1% penicillin/streptomycin and 24 mM HEPES buffer) at 37^0C in 5% CO_2. Cells were grown for 2-3 days and were allowed to reach 80% confluency. For the cell adhesion experiments, cells were harvested from culture flasks and suspended in supplemented DMEM. The cells were then counted using a haemocytometer, tested for cell viability using trypan blue and then diluted yielding a solution of 1 x 10^6 cells/ml.

Cell adhesion on different SAMs

The pure C11TEG and C3K-GRGDS:C11TEG mixed SAMs and bare gold substrates were done in triplicates and placed in sterile Petri dishes. The substrates were then immersed in 4 ml of supplemented DMEM. A 1 ml RAW 264.7 cell suspension of 1 x 10^6 cells/ml was then added to each Petri dish to give a final cell suspension of 2 x 10^5 cells/ml in each Petri dish. The substrates were incubated for 24 h at 37^{o}C in 5% CO_2. After incubation the substrates were rinsed in DMEM (warmed to 37^{o}C) to ensure loosely bound cells were removed. Substrates were then mounted on a microscope slide and the cells were counted using a microscope.

Cell switching studies

Electrical potentials were applied to the SAMs on Au using a Gamry PCI4/G300 with a custom designed Teflon cell, equipped with the functionalised Au substrate as the working electrode, a Pt wire as the counter electrode and a SCE as the reference electrode. For the bio-inactive state (cell resistant state), an electrical potential of -0.4 V was applied for 10 min on the gold substrate in 4 ml of DMEM, followed by adding a RAW 264.7 cell suspension of 1 x 10^6 cells/ml, whilst maintaining the -0.4 V potential for a further 1 h. The substrates were rinsed in DMEM warmed to 37^{o}C to ensure loosely bound cells were removed. Thereafter, 0.1 ml of trypan blue was added to the surface of the substrate that had undergone switching and the cell viability was checked under a microscope to ensure an applied potential of -0.4 V was not causing cell death. For the bioactive state (cell adhesive state), the same procedure was used but under OC conditions with no applied potential.

Cyclic voltammetry

Cyclic voltammetric studies were performed with a Gamry PCI4/G300 in a three-electrode Teflon cell consisting of a saturated calomel reference electrode (SCE), Pt wire counter electrode and the gold modified with the C3K-GRGDS:C11TEG mixed SAM as the working electrode. Gold surfaces modified with GRGDS:C11TEG mixed SAMs were placed in a petri dish with 4 ml of DMEM. A 1 ml aliquot of 4×10^6 cells were added and left to adhere to surfaces for 2 hours. Each substrate was placed in an electrochemical cell and cyclic voltammetry was performed from a starting potential of 0 V and switching potential of -0.4 V at a scan rate of 0.1 V/s.

Cell counting on surfaces

The cells were visualised with an optical microscope (Zeiss SM-LUX) using 20x objective lens. Images were collected with a Canon Powershot G5 monochrome camera. Cell numbers were quantified using cell counts on remaining cells per field of view at 5 random locations on each substrate.

X-ray Photoelectron Spectroscopy (XPS)

XPS spectra were obtained on the VG Escalab 250 instrument based at University of Leeds EPSRC Nanoscience and Nanotechnology Facility, UK. XPS experiments were carried out using a monochromatic Al K α X-ray source (1486.7 eV) and a take-off angle of 15°. High-resolution scans of S 2p, N 1s, C 1s and O 1s (Figure S2) were recorded using a pass energy of 150 eV at a step size of 0.05 eV. Fitting of XPS peaks was performed using the

Avantage V 2.2 processing software. Sensitivity factors used in this study were: S 2p, 2.08; N 1s, 1.73; C 1s, 1.00; O 1s 2.8; Au 4f 7/2, 9.58 and Au 4f 5/2, 7.54. The averages and standard errors reported were determined from at least four different XPS measurements. By integrating the area of the S 2 p and N 1s peaks for the mixed monolayers, we were able to calculate the ratio of C3K-GRGDS oligopeptide to C11TEG on the surface. The ratio calculation is based on the number of N and S atoms on each of the surfactants (C3K-GRGDS oligopeptide consists of 15 N and 1 S, whilst C11TEG has no N and 1 S). Based on that, the following equation can be used to calculate the number of C11TEG molecules per oligopeptide on the surface:

Number of C11TEG molecules per oligopeptide = (15 x S area/N area)-1) **Equation 1**

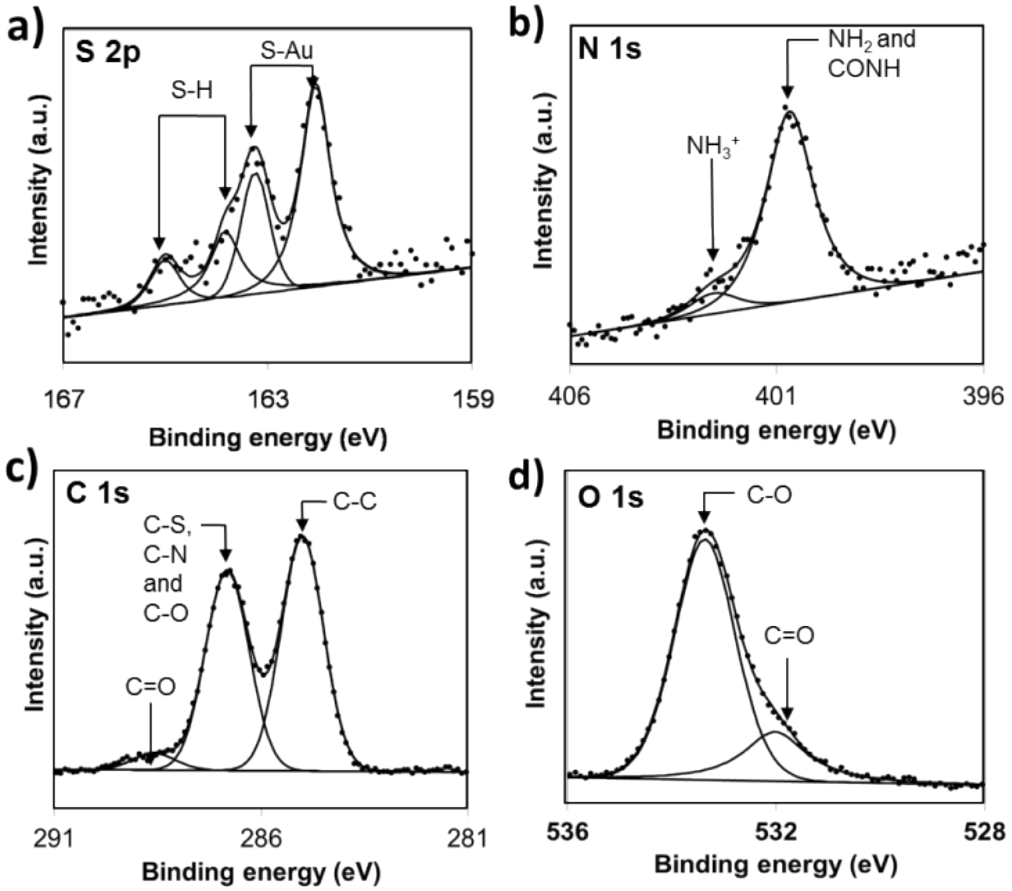

Figure S2. XPS spectra of the a) S 2p, b) N 1s, c) C 1s and d) O 1s peak regions of **C3K-GRGDS:C11TEG** mixed SAMs.

Cyclic voltammetric studies

Cyclic voltammetric studies were performed to demonstrate that no significant faradaic process occur over the potential range studied, and thus ions are not participating in redox reactions and consequently redox chemistry is not being significantly affected by application of the potential used . If redox chemistry was being there, it would be expected that we would see peak currents in the cyclic voltammograms indicative of a faradaic process. The macrophage cells were cultured on the **C3K-GRGDS:C11TEG** mixed SAM in DMEM medium and then placed in an electrochemical cell. The cyclic voltammograms (CVs) logged showed no significant faradaic processes from 0 to –0.4 V (Figure S3a), indicating that the potential used in our studies did not affect the redox constituents of the solution, and thus no impact on cells. It was also important to demonstrate that with increased time there were no faradic processes that occurred in the presence of cells. Therefore, 25 CVs were recorded and no change in redox chemistry was observed (Figure S3b).

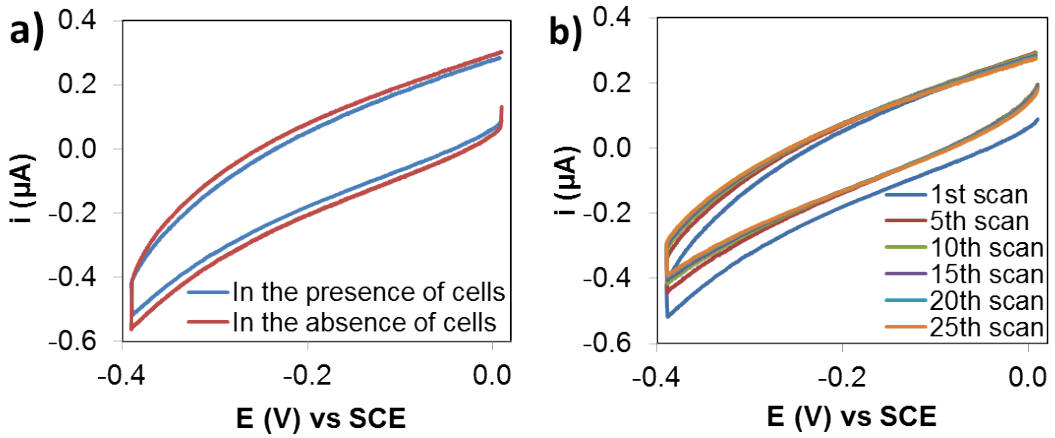

Figure S3. CVs obtained at a working electrode of gold modified with **C3K-GRGDS:C11TEG** mixed SAM a) in the presence and absence of adhered cells and b) during 25 consecutive cycles in the presence of adhered cells. CVs were performed at a scan rate of 0.1 V/s in PBS.

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References

(1) Wang, H.; Chen, S. F.; Li, L. Y.; Jiang, S. Y. Improved method for the preparation of carboxylic acid and amine terminated self-assembled monolayers of alkanethiolates. *Langmuir* **2005**, *21*, 2633-2636.