**README File:** Dynamic surfaces for the study of mesenchymal stem cell growth through adhesion regulation

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**Figure 1: Dynamic, enzyme cleavable surfaces for MSC growth**

**1A Schematic:** Cartoon representation of the FMOC and PEG blocked RGD surfaces illustrating the elastase cleavage site.

**1B ToF-SIMS:** TOF-SIMS image shows a uniform chemical surface composition on the micron scale and confirms that PEG, FMOC and amino acids (shown as the sum of the indicated representative peaks for each amino acid) are only detected in the analysis where expected.

ToF-SIMS analysis was carried out with a ToF-SIMS IV time of flight instrument (ION-TOF GmbH). Secondary ions were generated using a primary ion beam from a 25 kV Bi3+ liquid metal ion source with a 1 pA pulsed target current. The primary ion dose was kept below the static limit (less than 2.45 x 1012 ions per cm2). Built up surface charge was compensated using an electron gun producing a flux of low energy electrons (20 eV). Secondary ions were subjected to a post acceleration voltage of 10 kV and analysed with positive polarity with a single stage reflectron analyser.

Images were acquired by rastering the primary ion beam across the sample surface. On each sample, two small scale (500 μm x 500 μm; 2 μm resolution) and one large scale (3 mm x 3 mm; 10 μm resolution) areas were imaged. The data was processed using Surface Lab 6. Mass spectra were calibrated to known reference peaks from H+, CH3+, C2H5+, C3H7+ and C4H9+. Peaks from the samples were assigned to PEG, FMOC and the amino acids according to reference data from the literature. Ion intensity images were generated from these ions of interest by the software. For qualitative assessment of sample uniformity and presence of chemical functionalities, small scale images (500 μm x 500 μm) were used and the ions associated with amino acids were summed up and combined in a single image. The ion count scale for specific ion images (PEG, FMOC, amino acids) were manually adjusted to the same range for each sample to allow direct comparison. Total ion images were scaled individually.

For semi-quantitative assessment of the relative amount of FMOC removed from the surface, the large scale images (3 mm x 3 mm; one per sample type) were normalised to the total ion counts and divided into four regions of interest (ROI) that correspond to four equally sized, non-overlapping quadrants (1.5 mm x 1.5 mm) of the image. Normalised ion intensities for an FMOC related ion (C14H11+, m/z = 179) were generated for each ROI by the software to provide four datasets for each sample that were used to calculate a mean and standard deviation for the FMOC ion intensities before and after exposure of FMOC-AARGD and FMOC-FARGD to elastase. These normalised intensities were used to calculate a percentage decrease of FMOC on the two sample types. It should be noted that this method to generate numeric data of surface densities of chemical compounds is sensitive to small amounts of material on the surface but not fully quantitative due to a variety of factors affecting measured ion intensities from ToF-SIMS

File types (ion intensity values) were generated using propriety Surfacelab 6 software from ION-TOF. The resulting data was processed in excel to calculate averages, errors and sums of ion intensities. Origin and Excel were used to plot the graphs and a vector drawing program (Xara Xtreme Pro 5) to compile everything into the final figure. Dataset contains original data generated by Surfacelab 6.

**1C Live/Dead analysis:** MSCs were cultured on plain glass coverslips for 48 hours then treated with different concentrations of porcine pancreatic elastase (4.61 units/mg of protein) 1.0 - 0.1 mg/ml to determine optimum concentration (as per material and methods section). Cultures incubated with 0.1 mg/ml (0.461U) elastase were indistinguishable from controls that were cultured using standard culture media. Control cells were incubated for 15 minutes with methanol.

Live/Dead cytotoxicity kit (LIVE/DEAD® Viability/Cytotoxicity Kit, for mammalian cells) was used as per manufacturers instructions. Images where there are no cells present indicated that the elastase concentration detached the cells from the surface and these cells were lost during changeover of solutions. Dataset contains immunofluorescence images of cells stained with calcein AM (green = alive) or ethidium homodimer (red = dead) at the different concentrations of elastase.

**1D Solid-state fluorescence spectroscopy:** Surface-bound FMOC groups were seen to fluoresce at a wavelength of 315 nm and piperdine cleavage resulted in a loss of this signal. At 0.1 mg/ml it was seen that elastase cleaved the surface-bound FMOC groups also resulting in a loss of signal at 315 nm.

At each point during solid-phase peptide synthesis, the attachment of amino acids to the surface was monitored using solid-state fluorescence spectroscopy. The presence of a 315 nm peak indicates the successful attachment of Fmoc-protected amino acids while the absence of this peak indicates successful removal of the Fmoc moiety. The peak was also not present when plain glass, silane-coated and Peg18 coated samples were analysed. The Fmoc group was removed with piperidine prior to the attachment of successive amino acids. To reveal the underlying RGD peptide, elastase was used to digest the terminal alanine and adjacent Fmoc group. Spectra were recorded using a number of samples from each batch created at each successive amino acid attachment but not from the same coverslip each time. Samples were analysed at room temperature using a JASCO FP-6500 spectrophotometer (JASCO, JPN) with spectra manager™ software. Samples were attached to a glass microscope slide inserted into a custom-made rotatable holder within the spectrophotometer chamber. Samples were orientated at 30 degrees to the incident light to limit the amount of reflected excitation light hitting the detector. Excitation of the surface-tethered FMOC groups was carried out using an excitation wavelength of 270 nm with a slit width of 20 nm. Three spectra were recorded at each stage of synthesis using three different samples. Dataset contains numerical data for all substrate types obtained from solid-state fluoresce in CSV format.

**Figure 2: Dynamic control of MSC adhesion and tension**

Samples were washed in PBS and fixed in 10% formaldehyde/PBS for 15 minutes at 37°C. Cells were permeabilised at 4°C for 5 minutes (30 mM sucrose, 50 mM NaCl, 3 mM MgCl2.6H2O, 20 mM HEPES and 0.5% v/v Triton® X-100 in PBS adjusted to pH 7.2) and non-specific binding epitopes were blocked with 1% w/v BSA/PBS for 15 minutes at 37°C. The primary antibody (mouse monoclonal anti-vinculin IgG (1:150; Sigma) was diluted in PBS/BSA along with rhodamine-phalloidin (1:500; Molecular Probes). Samples were then incubated for 1 hour at 37°C after which they were washed in 0.5% v/v Tween 20/PBS (PBST, 3x 5 minutes under gentle agitation) to minimise background labelling. Horse biotinylated anti-mouse IgG (1:50; Vector Laboratories) in BSA/PBS was added to samples and incubated for 1 hour at 37°C. After washing stages, samples were incubated for 30 minutes at 4°C with fluorescein isothyiocyanate streptavidin (FITC; 1:50; Vector Laboratories) in BSA/PBS followed by a final washing stage. Coverslips were placed on glass slides in 4’6-diamidino-2-phenylindole (DAPI) mountant (Vector Laboratories).

For focal adhesion analysis, an average of 40 vinculin images (per substrate) were used to calculate the average adhesion number and length per cell. Data was processed using ImageJ to determine adhesion size and Excel to sort data into ascending order of size. This data was analysed using one-way annova (Prism software). Significant differences were determined using Dunn’s post hoc test. Graphical data is represented as a percentage of each sub-type compared to the total number of adhesions**.** For phosphomyosin analysis, an average of 40 phosphomyosin images (per substrate) were used to calculate phosphomyosin intensity. Data was processed using ImageJ (integrated density) and analysed using one-way annova (Prism software). Significant differences were determined using Dunn’s post hoc test. Dataset contains all immunofluorescence images for cells analysed for focal adhesion experiments and phosphomyosin experiments.

**Figure 3:** **Integrin, BMP2 receptor and cytoskeletal tethering changes in MSCs on dynamic surfaces.**

Samples were washed in PBS and fixed in 10% formaldehyde/PBS for 15 minutes at 37°C. Cells were permeabilised at 4°C for 5 minutes (30 mM sucrose, 50 mM NaCl, 3 mM MgCl2.6H2O, 20 mM HEPES and 0.5% v/v Triton® X-100 in PBS adjusted to pH 7.2) and non-specific binding epitopes were blocked with 1% w/v BSA/PBS for 15 minutes at 37°C. Primary antibodies (rabbit polyclonal anti Ezrin/Radixin/Moesin (Cell Signaling Technology; 1:50), rabbit polyclonal anti BMPR1A (Thermo Scientific; 1:50), mouse monoclonal anti-integrin β1 (Thermo Scientific; 1:50) or mouse anti-integrin αvβ5 (R&D system, 1:50)) were diluted in PBS/BSA along with rhodamine-phalloidin (1:500; Molecular Probes). Samples were then incubated for 1 hour at 37°C after which they were washed in 0.5% v/v Tween 20/PBS (PBST, 3x 5 minutes under gentle agitation) to minimise background labelling. Horse biotinylated anti-rabbit IgG (1:50; Vector Laboratories) or goat Texas red anti-rabbit IgG (Vector Laboratories) BSA/PBS was added to samples and incubated for 1 hour at 37°C. After washing stages, samples were incubated for 30 minutes at 4°C with fluorescein isothyiocyanate streptavidin (FITC; 1:50; Vector Laboratories) in BSA/PBS followed by a final washing stage. Coverslips were placed on glass slides in 4’6-diamidino-2-phenylindole (DAPI) mountant (Vector Laboratories). Datasets contain fluorescence images showing co-localisation of BMPR1a with Integrin **β**1 andIntegrin **β**5, and co-localisation of actin and ezrin.

RUNX2 analysis was carried out using in-cell western. Cells on substrates were washed with PBS and fixed with 10% v/v formaldehyde/PBS for 15 minutes at 37°C. Cells were permeabilised using pre-cooled methanol at 4°C for 10 minutes, and non-specific binding epitopes were blocked with 1% milk protein in 0.1% Tween 20/PBS (PBST) for 1 hour at room temperature. Cells were then washed three times (3x5 minutes) with 0.1% PBST. Primary antibodies diluted in 1% milk/PBST containing Cell Tag 700CW stain (1:500; LI-COR, Cat No: 926-41090) with rabbit polyclonal RUNX2 (Santa Cruz; 1:50) or rabbit polyclonal Phospho-RUNX2 pSer465 (Thermo Scientific; 1:50) for Phospho -RUNX2 expression in Ezrin knock-down cells. Cells with primary antibody were incubated at room temperature for 1 hour, and then washed with 0.1% PBST for three times. After washing, Cells with secondary antibody - either IRDye 800CW, Goat anti-mouse (LI-COR, Cat No: 926-32210) or Donkey anti-rabbit (LI-COR, Cat No: 926-32213) diluted in 1% milk/PBST at 1:1000 were incubated at room temperature for 1 hour or at 40C for overnight. After washing stage, cells were subjected to LI-COR Odyssey Sa (0157) scanner. The protein of interest was scanned at channel 800, and CellTag at channel 700 (scan parameters: Focus offset = 2.0mm, scan resolution = 100µm, intensity = 12). Data were collected in integrated intensity (IntegInten), and analysed by one-way analysis of variance (ANOVA) and Dunn’s post-hoc test applied where significance was determined as *p*<0.05.

Datasets contains immunofluorescence images for co-localisation studies and in-cell western readout for Ezrin and RUNX2.

**Figure 5: Analysis of MSC growth and differentiation at days 7 and 21.**

**5B** **STRO-1 quantification:** See Figure 2 for immunofluorescence methodology. MSCs were stained for STRO-1 (mouse monoclonal anti STRO-1 IgG (1:50; Insight Biotechnology)) and actin (rhodamine phalloidin (1:500)). An average of 50 fluorescence images were used to quantify the number of STRO-1 positive cells at day 7 and 21. Values were analysed in Excel and by using one-way analysis of variance (ANOVA) and Dunn’s post-hoc test to determine significant differences. Dataset contains immunofluorescence images of these samples.

**5C-D Osteopontin and Osteocalcin quantification:**  See Figure 2 for immunofluorescence methodology. MScs were stained for OPN (mouse monoclonal anti OPN IgG (1:50; Insight Biotechnology)) or OCN (mouse monoclonal anti OCN IgG (1:50; Insight Biotechnology)) along with actin (rhodamine phalloidin (1:500)). An average of 50 cells were analysed for each substrate and processed in ImageJ (integrated density) to determine the expression levels of each protein. Data was normalised against the number of cells (determined by DAPI staining) in each field of view. Dataset contains immunofluorescence images of these samples.

**Figure 4: Metabolite analysis of MSCs on dynamic surfaces.**

For metabolomics analysis, substrates were removed from the well plates and transferred to new sterile plates so that only cells that were attached to the substrates were used in the analysis. Substrates were washed once with warmed PBS then 0.5 ml of ice-cold extraction solvent (chloroform: methanol: water at 1:3:1 v/v) was added to the wells. Plates were sealed with parafilm to minimise evaporation and placed on a rotary shaker for 1 hour at 4°C. After this time the extraction solvent was transferred to sterile 0.5 ml eppendorfs and centrifuged at 13,000 g for 5 minutes to remove cell debris. The supernatant was transferred to LC vials otherwise samples were stored at -80°C in eppendorf tubes until use. For elastase studies, all samples were processed in-well with 150 µl of extraction solvent added to each well. Samples were then processed as above.

All samples were diluted 1 in 2 with acetonitrile prior to being aspirated to HPLC vials; an additional 5 µl of each sample was combined into a single aliquot to be used as a quality control sample. This pooled sample was injected several times throughout the duration of each run in order to monitor metabolite quality and sample degradation. Three standards containing a number of known metabolites were also run alongside unknown samples for the purpose of identifying all other metabolites. Chromatographic separation of metabolites was performed using an UltiMate 3000 RS-LC (Thermo Fisher) with a zwitterionic hydrophilic interaction liquid chromatography (ZIC-HILIC) column (C18 150 x 4.6mm; Merck Sequant) as the stationary phase, 1% v/v formic acid in acetonitrile as the organic mobile phase, and 1% v/v aqueous formic acid as the aqueous mobile phase. The mobile phase was run as a gradient over 46 minutes (table 1). Injection volumes were 10 µl and a ZIC-HILIC C8 20 x 2.0 guard column was used to protect the main column from impurities; chromatography columns were maintained at 25°C.

Raw data was processed in IDEOM/Excel interface and analysed using t-test with Bonferroni’s correction. Dataset contains raw files for each substrate tested and requires MetaboAnalyst software to analyse it.

**4A-C:** Putative metabolites were analysed using MetaboAnalyst 2.0 and the data was displayed as volcano plots relative to D2-plain (plain control at day 2).

**4D:** At 7 days (2 days ‘low’, 5 days ‘high’ for cleaved RGD), principle component analysis showed very clear metabolomics differences with MSCs on the FMOC-RGD (low) surface having a highly homogeneous metabolome, more so than on the controls, and the cleaved RGD (high) surfaces having a far more heterogeneous metabolome (n=3).

**4E:** Heatmap of putatively detected unsaturated lipids after 7 days culture showing up-regulation in MSCs on the FMOC-RGD surface, and down-regulation in MSCs on the cleaved RGD surface (n=3).

**4F-G:** Ingenuity® functional pathway analysis illustrating more, more significantly altered functional pathways in MSCs on the cleaved RGD surface (F) compared to those on FMOC-RGD surfaces (G). Functions include carbohydrate, small molecule, nucleic acid, lipid and

**Figure 6:** **Analysis of MSC adhesion and differentiation on PEG blocked surfaces.**

**6A-B** **Adhesion analysis at day 5 and STRO-1 at day 21 of culture:** See Figure 2 for immunofluorescence methodology. MSCs were cultured on PEG-blocked surfaces (PEG-RGD) or cleaved surfaces (Cleaved RGD) and stained for vinculin (mouse monoclonal anti-vinculin IgG (1:150; Sigma)) or STRO-1 (mouse monoclonal anti STRO-1 IgG (1:50; Insight Biotechnology)) and actin (rhodamine-phalloidin (1:500; Molecular Probes)). STRO-1 expression was analysed using ImageJ (integrated density) and normalised against the number of cells present (DAPI nuclear staining). Vinculin staining shows that MSCs developed longer adhesions (smAdhs) on PEG surfaces that had been treated with elastase to removed the PEG blocking group (not quantified). Dataset contains immunofluorescence images of cells on each of the two surfaces for both experiments.

**6C** **Principle component analysis from metabolomics data:** See Figure 4 for metabolomics methodology. Dataset contains metabomics raw data files which were processed in IDEOM/MzMatch excel interface. Principle component analysis (part of the MetaboAnalyst online metabolomics suite <http://www.metaboanalyst.ca>) was used to show that overall data was most homogeneous on PEG-RGD surfaces and most heterogeneous on the cleaved RGD surface as with the FMOC blocked samples.

**Supplementary figure 1:** **preparation of enzyme-responsive FMOC based substrates.**

Chemically cleaned glass coverslips (A) were modified with GOPTS solution (B). Addition of a PEG diamine provided the surface with amine functionality to enable subsequent coupling of amino acids (C). The complete surface structure was built up through stepwise coupling of amino acids and FMOC deprotection stages. As the amino acids were FMOC-protected the amino acid sequence naturally terminated in the FMOC blocking group (D). The structure also contains an Ala-Ala dipeptide that formed the designated enzyme-cleavable site. Enzymatic digestion resulted in the removal of the FMOC capping group and one of the alanine residues (E). R groups are (R1) CH2COOH for aspartic acid or CH2CH2COOH for glutamic acid, and (R2) is (CH2)3NHC(NH)(NH2) for arginine. This method was used for PEG-blocked substrates where the terminal FMOC group was removed with piperidine and replaced with PEG.

**Supplementary figure 2:** **Stepwise monitoring of peptide synthesis using solid-state fluorescence spectroscopy.**

At each point during solid-phase peptide synthesis, the attachment of amino acids to the surface was monitored using solid-state fluorescence spectroscopy. The presence of a 315 nm peak indicates the successful attachment of Fmoc-protected amino acids while the absence of this peak indicates successful removal of the Fmoc moiety. The peak was also not present when plain glass, silane-coated and Peg18 coated samples were analysed. The Fmoc group was removed with piperidine prior to the attachment of successive amino acids. To reveal the underlying RGD peptide, elastase was used to digest the terminal alanine and adjacent Fmoc group. Spectra were recorded using a number of samples from each batch created at each successive amino acid attachment but not from the same coverslip each time. Dataset contains numerical data for all substrate types obtained from solid-state fluoresce in CSV format.

**Supplementary figure 3:** **Water contact angle measurements.**

Three of each substrate was used and three droplets were analysed per substrate. Data contains the water contact angles for all substrates tested including left and right sides of the droplet, and the average of this value. The overall average and standard deviation was used to create the graph. For all CSV files containing Fmoc-amino acids, the first set of data refers to substrates where the Fmoc protecting group is in place while the second set of data refers to substrates where the Fmoc group has been chemically removed using 20% piperidine. Water contact angle (WCA) was carried out using the sessile drop technique with a KSV CAM 100 contact angle goniometer (KSV Instruments, USA). High contrast images of static water droplets were recorded and CAM 100 software was used to apply a circular fit to the droplet outline to determine contact angles across a series of measurements. A total of three droplets were recorded per surface (25 frames per droplet) and three of each surface used. Averages were pooled for the main data. Dataset contains numerical data for all substrates at each stage for WCA in CSV format.

**Supplementary figure 4: Analysis of 0.1 mg/ml elastase on cell responses.**

**4A MTT:** After 5 days, 100 µl of 5 mg/ml MTT (methylthiazolyldiphenyl-tetrazolium bromide) solution in complete cell culture media was added to the cells for 2 hours. After this time, the media was removed and replaced with DMSO for 5 minutes. Liquid from each well was then analysed at 570 nm (background set to 670 nm) and optical density reported.

Control cultures were incubated with standard culture media while elastase-containing cultures were incubated with 0.1 mg/ml elastase. Dataset contains readout from the plate reader and analysis in excel.

**4B Metabolomics:** For metabolomics analysis, substrates were removed from the well plates and transferred to new sterile plates so that only cells that were attached to the substrates were used in the analysis. Substrates were washed once with warmed PBS then 0.5 ml of ice-cold extraction solvent (chloroform: methanol: water at 1:3:1 v/v) was added to the wells. Plates were sealed with parafilm to minimise evaporation and placed on a rotary shaker for 1 hour at 4°C. After this time the extraction solvent was transferred to sterile 0.5 ml eppendorfs and centrifuged at 13,000 g for 5 minutes to remove cell debris. The supernatant was transferred to LC vials otherwise samples were stored at -80°C in eppendorf tubes until use. For elastase studies, all samples were processed in-well with 150 µl of extraction solvent added to each well. Samples were then processed as above.

All samples were diluted 1 in 2 with acetonitrile prior to being aspirated to HPLC vials; an additional 5 µl of each sample was combined into a single aliquot to be used as a quality control sample. This pooled sample was injected several times throughout the duration of each run in order to monitor metabolite quality and sample degradation. Three standards containing a number of known metabolites were also run alongside unknown samples for the purpose of identifying all other metabolites. Chromatographic separation of metabolites was performed using an UltiMate 3000 RS-LC (Thermo Fisher) with a zwitterionic hydrophilic interaction liquid chromatography (ZIC-HILIC) column (C18 150 x 4.6mm; Merck Sequant) as the stationary phase, 1% v/v formic acid in acetonitrile as the organic mobile phase, and 1% v/v aqueous formic acid as the aqueous mobile phase. The mobile phase was run as a gradient over 46 minutes (table 1). Injection volumes were 10 µl and a ZIC-HILIC C8 20 x 2.0 guard column was used to protect the main column from impurities; chromatography columns were maintained at 25°C.

Control cultures were incubated with standard culture media while elastase-containing cultures were incubated with 0.1 mg/ml elastase. Metabolic changes showed little difference in metabolites between control and elastase cultures. Raw data was processed in IDEOM/Excel interface and analysed using t-test with Bonferroni’s correction. Dataset contains raw files for each substrate tested and requires MetaboAnalyst software to analyse it.

**4C Immunofluorescence:** Samples were washed in PBS and fixed in 10% formaldehyde/PBS for 15 minutes at 37°C. Cells were permeabilised at 4°C for 5 minutes (30 mM sucrose, 50 mM NaCl, 3 mM MgCl2.6H2O, 20 mM HEPES and 0.5% v/v Triton® X-100 in PBS adjusted to pH 7.2) and non-specific binding epitopes were blocked with 1% w/v BSA/PBS for 15 minutes at 37°C. Primary antibodies (mouse monoclonal anti-phosphomyosin IgG (1:200; Cell Signaling Technology), rabbit polyclonal anti RUNX2 (1:50; Insight Bio) or mouse monoclonal anti vimentin (1:50; Sigma)) were diluted in PBS/BSA along with rhodamine-phalloidin (1:500; Molecular Probes). Samples were then incubated for 1 hour at 37°C after which they were washed in 0.5% v/v Tween 20/PBS (PBST, 3x 5 minutes under gentle agitation) to minimise background labelling. Horse biotinylated anti-mouse IgG (1:50; Vector Laboratories) or horse biotinylated anti-rabbit IgG (Vector Laboratories) BSA/PBS was added to samples and incubated for 1 hour at 37°C. After washing stages, samples were incubated for 30 minutes at 4°C with fluorescein isothyiocyanate streptavidin (FITC; 1:50; Vector Laboratories) in BSA/PBS followed by a final washing stage. Coverslips were placed on glass slides in 4’6-diamidino-2-phenylindole (DAPI) mountant (Vector Laboratories).

Control cultures were incubated with standard culture media while elastase-containing cultures were incubated with 0.1 mg/ml elastase. Dataset contains fluorescence images showing cell adhesion and spread in both conditions.

**Supplementary figure 5: ToF-SIMS analysis to confirm RGD is left on the surface after elastase cleavage.**

ToF-SIMS analysis was carried out with a ToF-SIMS IV time of flight instrument (ION-TOF GmbH). Secondary ions were generated using a primary ion beam from a 25 kV Bi3+ liquid metal ion source with a 1 pA pulsed target current. The primary ion dose was kept below the static limit (less than 2.45 x 1012 ions per cm2). Built up surface charge was compensated using an electron gun producing a flux of low energy electrons (20 eV). Secondary ions were subjected to a post acceleration voltage of 10 kV and analysed with positive polarity with a single stage reflectron analyser.

Images were acquired by rastering the primary ion beam across the sample surface. On each sample, two small scale (500 μm x 500 μm; 2 μm resolution) and one large scale (3 mm x 3 mm; 10 μm resolution) areas were imaged. The data was processed using Surface Lab 6. Mass spectra were calibrated to known reference peaks from H+, CH3+, C2H5+, C3H7+ and C4H9+. Peaks from the samples were assigned to PEG, FMOC and the amino acids according to reference data from the literature. Ion intensity images were generated from these ions of interest by the software. For qualitative assessment of sample uniformity and presence of chemical functionalities, small scale images (500 μm x 500 μm) were used and the ions associated with amino acids were summed up and combined in a single image. The ion count scale for specific ion images (PEG, FMOC, amino acids) were manually adjusted to the same range for each sample to allow direct comparison. Total ion images were scaled individually.

For semi-quantitative assessment of the relative amount of FMOC removed from the surface, the large scale images (3 mm x 3 mm; one per sample type) were normalised to the total ion counts and divided into four regions of interest (ROI) that correspond to four equally sized, non-overlapping quadrants (1.5 mm x 1.5 mm) of the image. Normalised ion intensities for an FMOC related ion (C14H11+, m/z = 179) were generated for each ROI by the software to provide four datasets for each sample that were used to calculate a mean and standard deviation for the FMOC ion intensities before and after exposure of FMOC-AARGD and FMOC-FARGD to elastase. These normalised intensities were used to calculate a percentage decrease of FMOC on the two sample types. It should be noted that this method to generate numeric data of surface densities of chemical compounds is sensitive to small amounts of material on the surface but not fully quantitative due to a variety of factors affecting measured ion intensities from ToF-SIMS

File types (ion intensity values) were generated using propriety Surfacelab 6 software from ION-TOF. The resulting data was processed in excel to calculate averages, errors and sums of ion intensities. Origin and Excel were used to plot the graphs and a vector drawing program (Xara Xtreme Pro 5) to compile everything into the final figure. Dataset contains original data generated by Surfacelab 6.

**Supplementary figure 6:** **Changing peptide sequence to alter elastase addressability.**

See Supplementary figure 5. Dataset contains original data generated by Surfacelab 6.

**Supplementary figure 7:** **Live/dead viability assay.**

Cells were cultured on plain controls, PEG18, RGD and RGE controls, FMOC-RGD and FMOC-RGE for 24h in α-MEM. Dead control cells were prepared by adding 70% ethanol to cells cultured on plain coverslips after 24h. Culture media was removed and cells were washed twice in warm phosphate-buffered saline (PBS), which was then replaced with live/dead assay reagents (2 μM calcein AM and 4 μM ethidium homodimer-1 in PBS), and incubated at room temperature until colour development occurred (approximately 15 mins in the dark). The stain was removed and the cells were rinsed twice in warm PBS. Dataset contains immunofluorescence images demonstrating live and dead cells in culture.

**Supplementary figure 8:** **Stro1 MSCs seeded at a density of 7 cells/mm2.**

Dataset contains light microscopy images of cells taken at 24 hours and 7 days showing the morphology post seeding. The images show that cell adhesion and degree of spreading was different across the substrates with MSCs cultured on PEG18, ARGE, Fmoc-D/E and Dige-E substrates initially struggling to attach and spread at 24 hours.

**Supplementary figure 9: Quantification of MSC size using different seeding densities.**

See Supplementary figure 4C for immunofluorescence methodology. For each seeding density (7/39/75 cells/mm^2), an average of 40 actin images were used to calculate cell size using ImageJ. Data was analysed using one-way annova (Prism software) and significant differences determined using Dunn’s post hoc test. Dataset contains immunofluorescence images of all cells analysed on each of the surfaces.

**Supplementary figure 10:** **A complete list of significant differences for Stro1 MSC size at 7 cells/mm2.**

Dataset contains a list of significant difference for all samples tested.

**Supplementary figure 11:** **A complete list of significant differences for MSC adhesion analysis.**

Dataset contains a list of significant difference for all samples tested.

**Supplementary figure 12: Cell proliferation**

**12A BrdU:** Cells were cultured on plain glass coverslips, plain controls, PEG18, RGD and RGE controls, FMOC-RGD and FMOC-RGE substrates in α-MEM, and pulse-labelled with 10 μM 5-bromo-2-deoxyuridine (BrdU) for 4h prior to fixation for 1 day and 4 day time points. Samples were washed in PBS and fixed in 10% formaldehyde/PBS for 15 minutes at 37°C. Cells were permeabilised at 4°C for 5 minutes (30 mM sucrose, 50 mM NaCl, 3 mM MgCl2.6H2O, 20 mM HEPES and 0.5% v/v Triton® X-100 in PBS adjusted to pH 7.2). The primary antibody (Mouse monoclonal anti-BrdU (clone BU-1, 1:100 in nuclease solution, prepared according to the manufacturer’s instructions in kit RPN202; GE Healthcare) was diluted in PBS/BSA. Samples were then incubated for 1.5 hours at 37°C after which they were washed in 0.5% v/v Tween 20/PBS (PBST, 3x 5 minutes under gentle agitation) to minimise background labelling. Horse biotinylated anti-mouse IgG (1:50; Vector Laboratories) and rhodamine-phalloidin (1:500; Molecular Probes) in BSA/PBS was added to samples and incubated for 1 hour at 37°C. After washing stages, samples were incubated for 30 minutes at 4°C with fluorescein isothyiocyanate streptavidin (FITC; 1:50; Vector Laboratories) in BSA/PBS followed by a final washing stage. Coverslips were placed on glass slides in 4’6-diamidino-2-phenylindole (DAPI) mountant (Vector Laboratories). Dataset contains immunofluorescence images of cells with BrdU positive and negative cells counted in Excel to determine the percentage of positive BrdU cells.

**12B Average number of cells per FOV:** For each substrate, 20 random DAPI images were used to calculate the average number of nuclei per field of view as an indicator of cell numbers with respect to each surface. Data was processed using ImageJ and analysed using one-way annova (Prism software). Significant differences were determined using Dunn’s post hoc test. Dataset contains raw DAPI images used to count the number of cells.

**Supplementary figure 13:** **Immunofluorescence images of Stro1 MSCs at day 7.**

Samples were washed with PBS and fixed with 10% v/v formaldehyde/PBS for 15 minutes at 37°C as per section 3.2.6. Cultures immuno-labelled for OPN and OCN were permeabilised at 4°C for 5 minutes; all samples were treated with 1% w/v BSA/PBS for 15 minutes at 37°C to block non-specific binding epitopes. Primary antibodies were diluted in BSA/PBS to make up solutions of rhodamine-phalloidin (1:500) with either mouse monoclonal anti-STRO-1 IgM (1:50), rabbit polyclonal anti-ALCAM IgG (1:50), mouse monoclonal OPN IgG (1:50), or mouse monoclonal OCN IgG (1:50). Samples were incubated for 1 hour at 37°C and rinsed with 0.5% v/v PBST (3x 5 minutes under agitation) to minimise background labelling. Horse biotinylated anti-mouse IgG (1:150) in BSA/PBS was added to STRO-1, OPN and OCN samples, and horse biotinylated anti-rabbit in BSA/PBS was added to ALCAM samples. All samples were incubated for 1 hour at 37°C then washed with PBST. After washing, samples were incubated for 30 minutes at 4°C with FITC (1:50) in BSA/PBS, followed by a final wash. Coverslips were placed on glass slides in DAPI mountant and cells were imaged with a fluorescence microscope. For each substrate, a number of immunofluorescence images were used to identify MSC (STRO-1/ALCAM) or Osteo (OPN/OCN) -specific markers. For osteopontin (OPN) and osteocalcin (OCN) expression, images were processed in ImageJ to obtain integrated densities. Data was analysed using one-way annova (Prism software) and significant differences were determined using Dunn’s post hoc test. STRO-1 and ALCAM were not quantified using these images. Dataset contains immunofluorescence images of actin with corresponding OPN/OCN/STRO-1 and ALCAM, and DAPI.

**Supplementary figure 14: Osteocalcin image analysis at 3 and 5 days culture.**

For each substrate, the entire sample was scanned using a scanning fluorescence microscope to obtain osteocalcin images at day 1, 3 and 5 with Plain day 1 used as a control sample. Data was processed using ImageJ and analysed using one-way annova (Prism software). Significant differences were determined using Dunn’s post hoc test. Dataset contains individual fluorescence images for osteocalcin. These images stitch together to show the whole surface.

**Supplementary figure 15: Induction of differentiation after 21 days of culture on FMOC-RGD.**

Cells were cultured for 2 weeks on plain controls and FMOC-RGD surfaces in standard α-MEM. After this time, samples were divided into 3 groups; control groups cultured in standard α-MEM, osteogenic cultures incubated with osteogenic induction media (350 μM ascorbate-2-phosphate, 0.1 μM dexamethasone in DMEM with 10% FBS), and adipogenic cultures that were alternated between adipogenic induction media (1 μM dexamethasone, 1.7 nM insulin, 200 μM indomethacin, 500 μM isobutylmethylxanthine in DMEM with 10% FBS, L-glutamine and antibiotics) and maintenance medium (1.7 nM insulin in DMEM with 10% FBS, 200 mM L-glutamine and antibiotics). The 3 groups were cultured for 4 weeks before fixation. Dataset contains immunofluorescence images of cells cultured in the different conditions and stained for FABP and OCN.

**Supplementary figure 16: Expression of adipogenic and chondrogenic phenotypes after 14 days of culture.**

See Supplementary figure 4C for immunofluorescence methodology. MSCs were cultured on each substrate and assessed using immunofluorescence for the presence of chondrogenic (rabbit polyclonal anti collagen II IgG; Insight Bio 1:50) and adipogenic (rabbit polyclonal anti fatty acid binding protein igG; Abcam 1:50) protein markers. Dataset contains immunofluorescence images of all cells analysed for this experiment. Data was not quantified.

**Supplementary figure 17:** **Analysis of MSC growth and differentiation at day 28 – Immunofluorescence images of STRO-1 and OCN.**

For each substrate, a number of immunofluorescence images were used to identify osteocalcin expression. Images were processed in ImageJ to obtain integrated densities and data was analysed using one-way annova (Prism software). Significant differences were determined using Dunn’s post hoc test. Dataset contains immunofluorescence images for all cells and substrates analysed.

**Supplementary figure 18:** **Network analysis of metabolites affecting growth-related biochemistry – FMOC-RGD and Cleaved RGD.**

See Supplementary figure 4B for metabolomics methodology. Dataset contains metabolomics raw data files which were analysed in Ingenuity(R) Pathway Analysis (IPA) to generate metabolite networks for ‘off’ FMOC-RGD versus ‘on’ (cleaved RGD) substrates.

**Supplementary figure 19:** **Network analysis of metabolites affecting growth-related biochemistry – FMOC-RGD and Cleaved-RGD.**

See Supplementary figure 18. Dataset contains raw files for use with MetaboAnalyst and other metabolomics software.

**Supplementary figure 20: STRO-1 image analysis for long-term growth before switch.**

See Supplementary figure 13 for immunofluorescence methodology. MSCs were cultured for 4 weeks (2 week ‘low’ and 2 week ‘high’ for cleaved RGD) and stained for STRO-1. ImageJ software (integrated density) was used to determine the amount of STRO-1 present and normalised against the number of cells present in the image. Statistics were processed by one-way ANOVA and Dunn’s post hoc test to determine significant differences. Dataset contains immunofluorescence images of cells that were analysed in this experiment.