Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information for:

Decorated Self-Assembling β^3-Tripeptide Foldamers Form Cell Adhesive Scaffolds

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Peptide Synthesis and Purification

All peptides were synthesized on a 100 µmol scale using standard Fmoc chemistry on Wang resin (0.9 mmol/g loading, GL Biochem, Shanghai, China). The resin was washed (3 x 30 s) with DMF and the Fmoc-protected β-amino acid (3.1 eq. to resin loading) was dissolved in DMF along with HBTU (3 eq. to resin loading), HOBt (3 eq. to resin loading) and DIPEA (4.5 eq. to resin loading). 4-Dimethylaminopyridine (0.1 eq. to resin loading) in DMF was added dropwise and the reaction proceeded overnight.

At this stage peptide synthesis of all peptides proceeded as normal. Following the overnight coupling, the resin was washed with DMF (5 x 30 s) and DCM (5 x 30 s) and peptide synthesis was continued. One cycle of peptide elongation consisted of the following steps. The loaded resin was first washed with DMF (3 x 30 sec) and the terminal Fmoc protecting group was removed with 20% piperidine/DMF (2 x 15 min). The deprotected resin was then washed with DMF (5 x 30 s) and treated for 90 min with a solution containing 3.1 eq. of the appropriate β-amino acid, 3 eq. HBTU, and 4.5 eq. DIPEA. The resin was then washed three times with DMF (3 x 30 s), unreacted amino groups were acetylated upon treatment with 10% v/v acetic anhydride and 1% v/v DIPEA in DMF (2 x 20 min), and the capped resin washed with DMF (3 x 30 s). These steps were repeated until the β-peptide sequence was complete. Once the final Fmoc-protecting group had been removed, the resin was treated with 10% v/v acetic anhydride and 1% v/v DIPEA in DMF (2 x 20 min) to afford an acetyl-capped N-terminus. The resin was subsequently washed with DMF (5 x 30 s) and DCM (5 x 30 s), dried for 20 min under vacuum, and then treated for 90 min with a cleavage solution containing 2.5% v/v water and 2.5% v/v triisopropylsilane in TFA. The cleaved resin was washed twice with the cleavage solution (2 x 30 s) and the cleaved β-peptide in TFA was collected. The TFA was evaporated under a stream of N2 and the peptide was precipitated by the addition of diethyl ether. The precipitate was filtered through a sintered glass funnel and reconstituted in H2O/acetonitrile (1:1) for lyophilization.

To functionalise an amino acid side-chain, the diaminobutyric acid 'Fmoc-L-Dbu(N3)-OH' (further referred to as 'β-azidohomoalanine') was used in synthesis which was reduced as previously described (accepted pending revisions, Motamed et. al., Soft Matter, 2016). Briefly, triphenylphosphine (3 eq. to resin loading) was dissolved in 4 ml tetrahydrofuran with 2.5% (v/v) mq-H_2O and added to the resin. Microwave irradiation was initiated at 60 °C, 100 Watt, 75 Psi for 2 h in a Microwave Peptide Synthesizer (CEM Corporation). The resin was washed with 95% (v/v) THF/5% (v/v) mq-H2O and 5x DMF before coupling of the next amino acid as previously described.

The success of each synthesis was assessed first by HPLC and ESI-MS analysis of the crude reaction mixture. Peptides were then purified to homogeneity by reverse-phase HPLC. The identities and purities of purified peptides were assessed by analytical HPLC with a solvent gradient of 0-50% 0.1% acetonitrile over 50 min and by mass spectrometry. Previous studies have shown that the TFA counter-ion associated with the peptide, which is a product of the

final step of synthesis, is toxic to cells and was therefore exchanged for a chloride salt by repeated lyophilisation in 0.1M HCl. Peptides were therefore salt exchanged with the Cl ion and used for all *in vitro* experiments.

Table 1: List of all peptides synthesised with MS data

Name	Sequence	MS calc.	MS obs.
A	Ac-βS-βAz(αIKVAV)βA	898.4	899.4 (+ve)
B	Ac-βS-βAz(αRGD)βA	700.2	701.2 (+ve)
C	Ac-βL-βI-βA	399.1	398.1 (-ve)

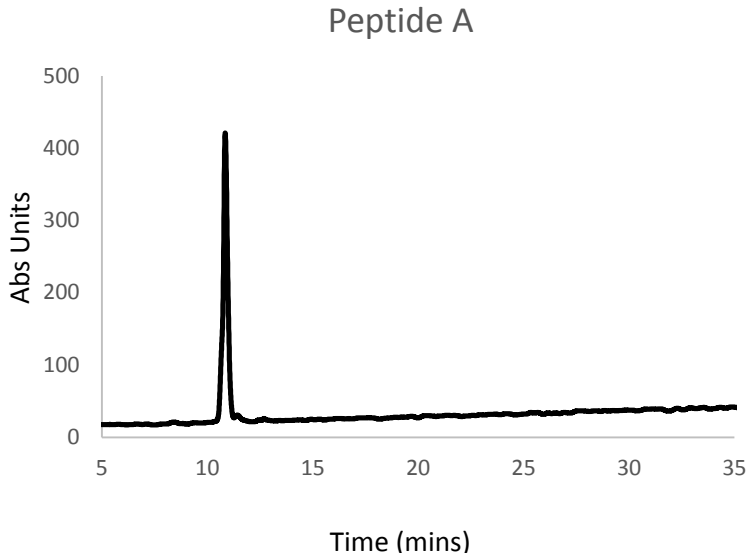

Figure SI-1: HPLC trace of peptide **A**

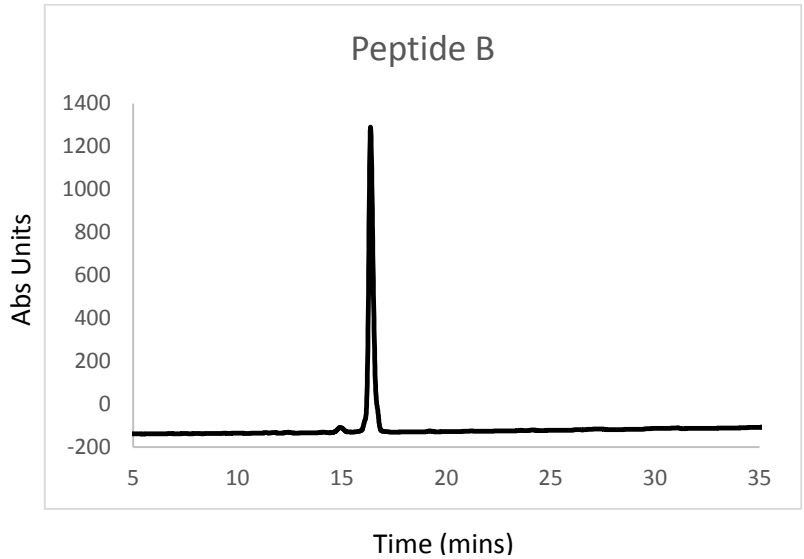

Figure SI-2: HPLC trace of peptide **B**

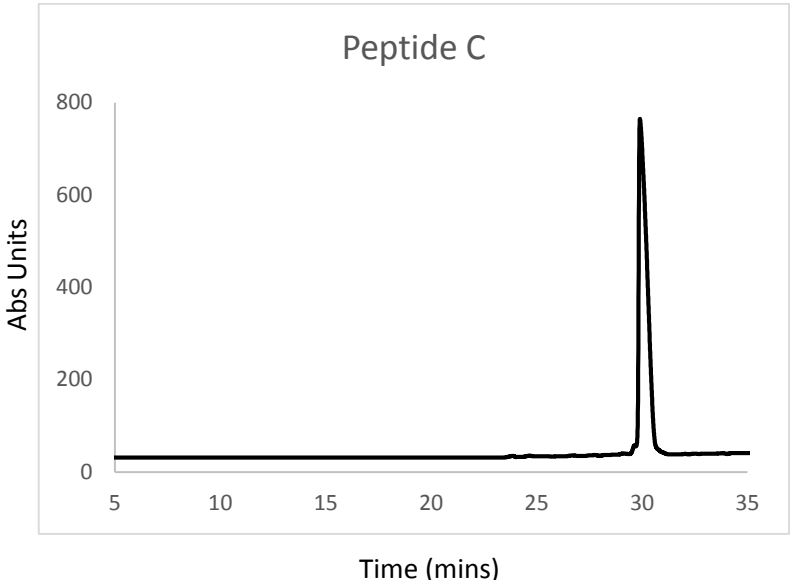

Figure SI-3: HPLC trace of peptide **C**

Circular Dichroism

CD measurements for all peptides were performed using a Jasco J-815 Circular Dichroism Spectropolarimeter (Jasco Corp., Japan). Secondary spectra for all peptides were obtained between a wavelength of 190-260nm. The following parameters were used; a scan speed 50nm/min, bandwidth 1.0 nm, resolution 1 nm with a 1 second response. A 0.1 mm quartz cuvette was used in which five repeat scans were compiled to generate the average spectra. β-peptides were dissolved in water to a concentration of 0.05mg/mL. The quartz cell temperature of 20 ˚C was maintained using a thermostatic water bath and stabilised using a Peltier temperature controller. The results were evaluated using the Jasco Spectra Manager and results shown in Fig SI-4.

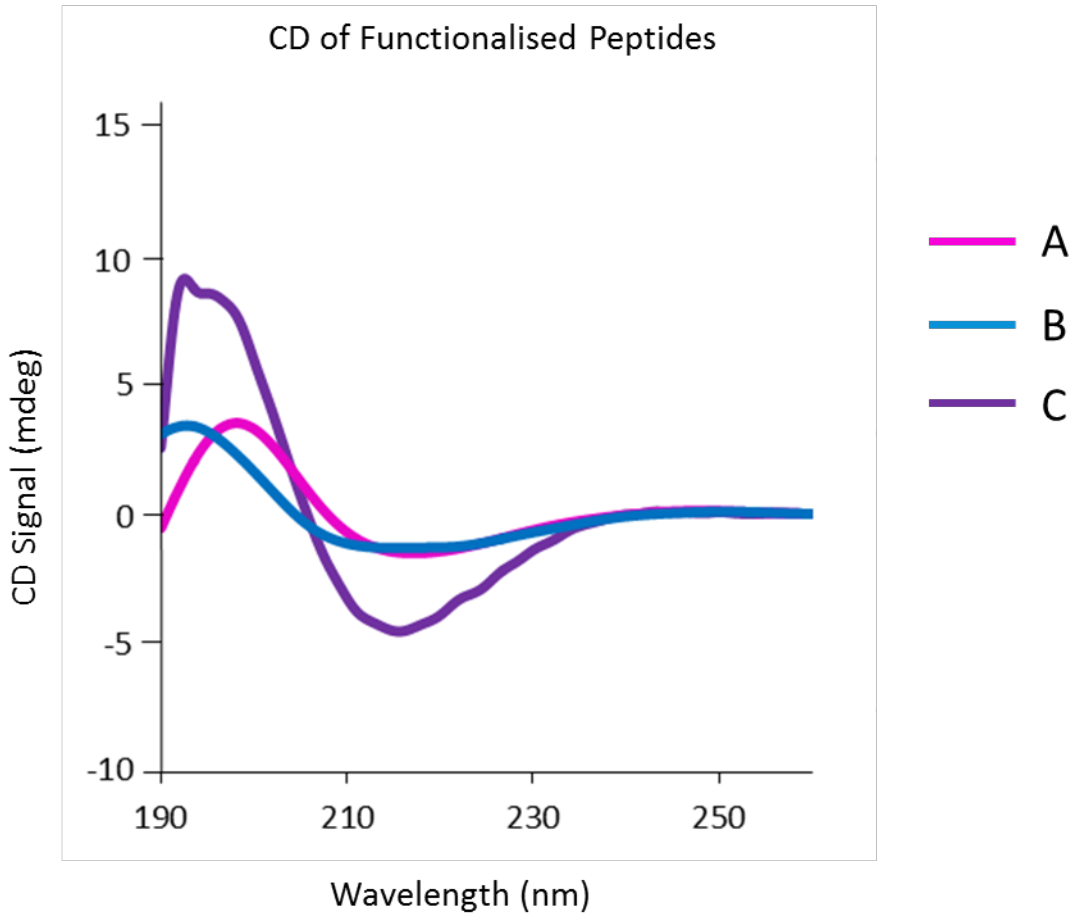

Fig SI-4: CD spectra of peptides **A**, **B** and **C** in water indicating the peptides adopt a 14-helical structure.

Atomic Force Microscopy

Peptide samples were deposited onto freshly cleaved mica, either undiluted (1.0-1.5 µl) or diluted directly on the surface of the mica (5 µl of solvent + 1 µl of sample). The solution was air-dried overnight and imaged using the Atomic force microscope NanoScope® IV with a MultiMode™ head (Veeco), equipped with an 'E-scanner' or 'J-scanner' and SPM probes 'NSC15/AlBS' (MikroMasch) with a resonance frequency of 325 kHz and spring constant 40 N/m. Images were produced in tapping mode at scan sizes of 50 µm x 50 µm, 10 µm x 10 µm or 3 µm x 3 µm and processed using software 'Gwyddion 2.38'.

The measurement of fibre height was done using SPM data visualization and Gwyddion 2.38 where a sample of ten fibres was selected. From these positions, profiles were extracted to Microsoft Excel to produce normalized fibre profile graphs.

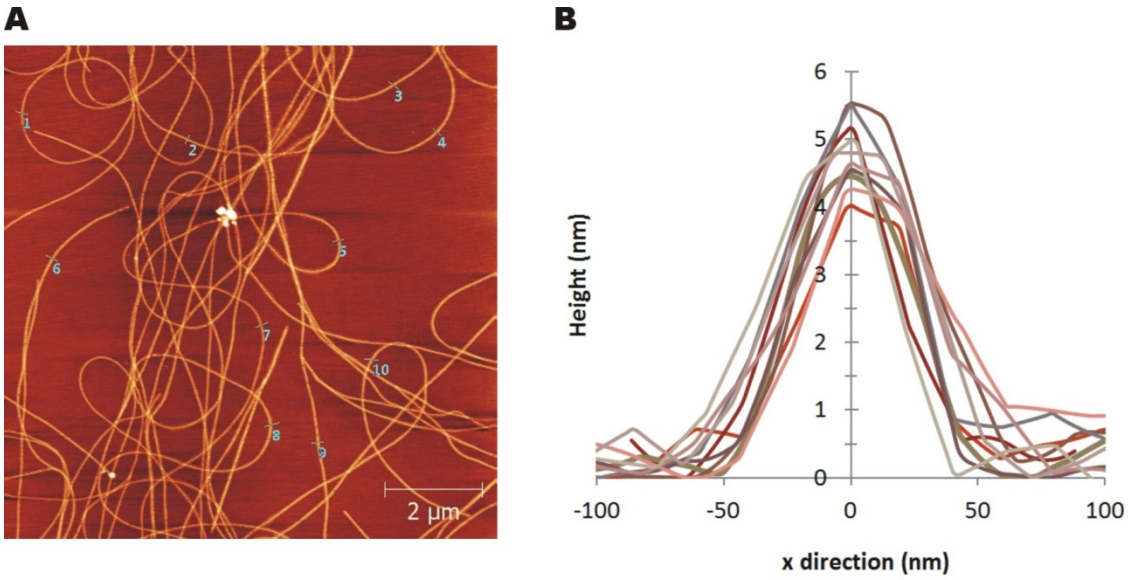

Figure SI-5: Height analysis of fibres formed by peptide **A** showing uniformity in height by (A) AFM and (B) these fibres were measured to be between 4 and 5 nm.

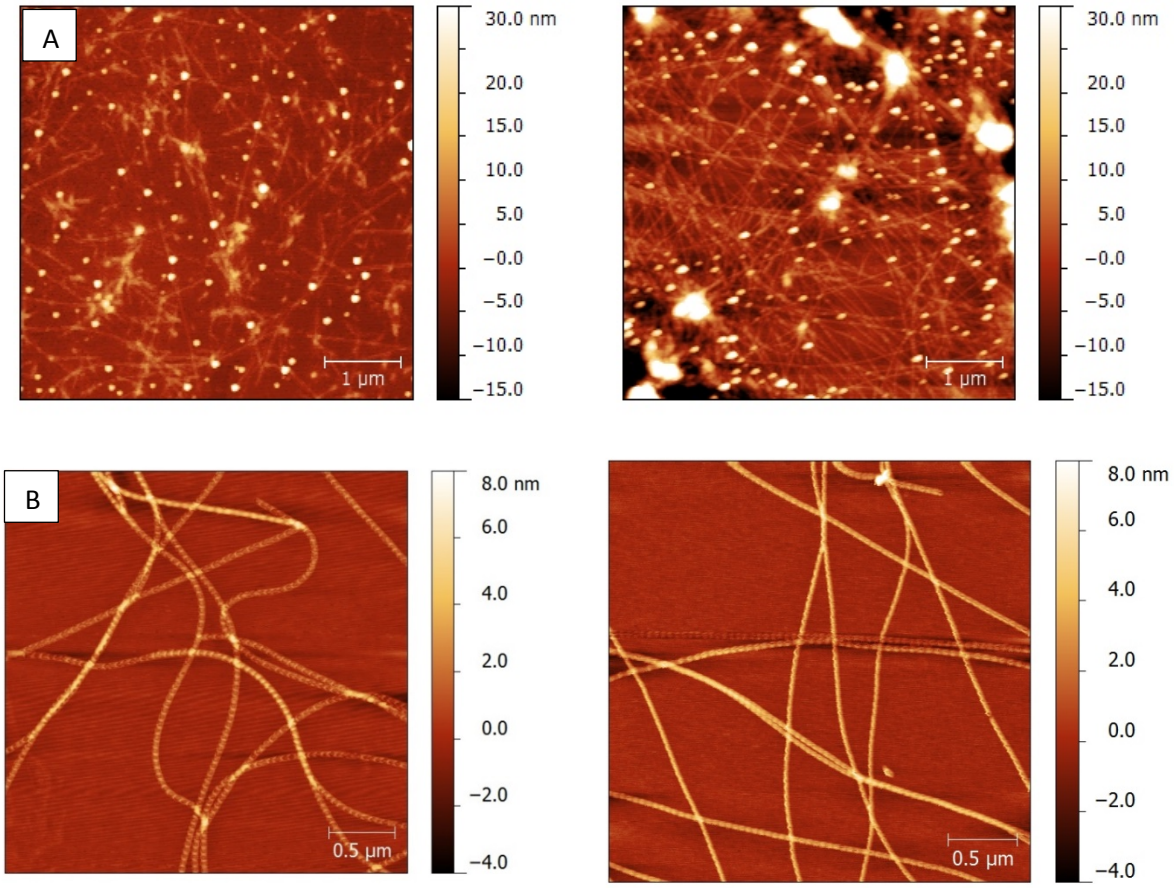

Figure SI-6: AFM images of fibres formed from peptide A. Panel A showing self-assembly of peptide **A** at (i) room temperature overnight or (ii) incubation at 37°C overnight. Panel B showing the self-assembled structures formed by peptide **A** as (i) a TFA salt or (ii) a Cl salt and were incubated in water for 1 week.

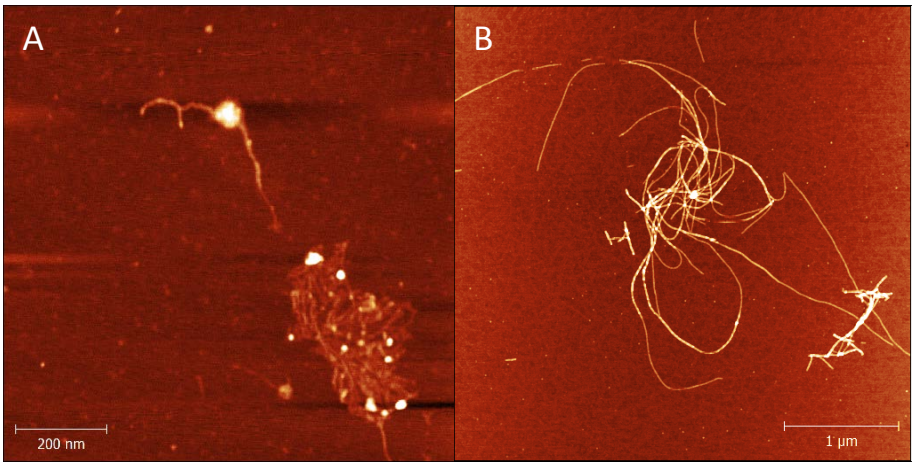

Figure SI-7: AFM images of peptide **B** following incubation in water for (A) 1 day or (B) 7 days

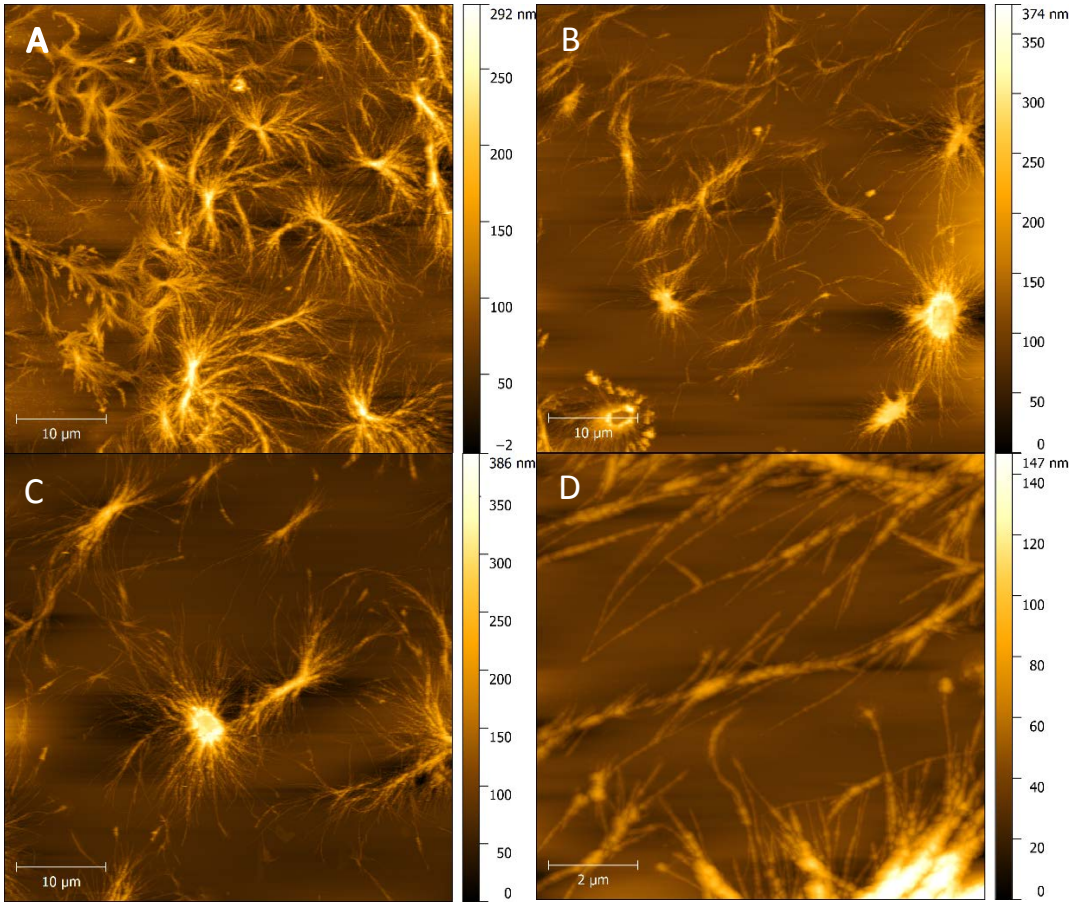

Figure SI-8: AFM images showing the extent of dendritic structure present in (A) peptide **C** alone compared to (B) mixture of peptides **A** and **C** (1:4); (C) mixture of peptides **B** and **C** (1:4) and (D) mixture of peptides **A, B** and **C** (1:1:3). These images show the significant reduction in the number of dendritic structures when peptide **C** is combined with either peptide **A** or **B** or both **A** and **B**.

Transmission Electron Microscopy

A 10 µl drop of solution containing peptide was placed onto parafilm. A formvar-coated grid was placed face down on the solution and left for five minutes. The sample was gently blot dried and left to dry further for 30 min. The samples were imaged using a Hitachi H6500 Transmission Electron Microscope.

Cell culture

Adult primary cardiac fibroblasts were isolated from C57/BL6 mice of around 10-12 weeks age. Mice were anesthetized with isofluorane, the heart was removed and placed in sterile PBS. Residual blood was ejected thoroughly and then atrium and blood vessels were cut. After washing 2x with PBS the heart was minced and treated with 0.1 % collagenase type I-S in HANKS/0.125% trypsin in PBS in the presence of agitation at 37 °C for 2x 30 mins and a third time with 0.125% trypsin/EDTA for 30min. Cells were then filtered through a 70 µm mesh net and neutralized with DMEM. Following a centrifugation at 1300 rpm for 5 min, the pellet was re-suspended in 5 ml DMEM and transferred to a Falcon® T25 tissue culture flask (Corning). Cells were incubated at 37 °C in a humidified atmosphere of 95% air and 5% CO_2 and after 1.5 hours media was changed to select for cardiac fibroblasts. Primary cardiac fibroblasts were maintained in DMEM and incubated at 37 °C and 5 % CO_2 in air. At sub-confluence cells were sub-cultured using 0.25 % trypsin/EDTA. For all experiments, cells from first or second passage were used.

The immortalized cell line bone marrow-derived macrophages (BMDMs) were grown in DMEM and kept in 37 °C with 5% CO_2. The medium was changed two to three times a week and cells were sub-cultured by scraping with Falcon® cell scraper (Corning) upon being 90% confluent.

Cell adhesion assays

Decorated β-tripeptides were incubated at a concentration of 1 mg/ml in sterile H_2O for 1 week at 37 °C to allow for optimum fibre formation. The presence of fibres was checked by AFM and the sample was then diluted to the appropriate plating concentrations (0.5 mg/ml). A volume of 25 µl was plated into wells of 96-well Microtest™ flat-bottom, polystyrene tissue culture plates (Becton Dickinson) and solution was air-dried in the tissue culture hood. Plates with peptides were stored for up to 2 weeks at 4 °C and UV-sterilized prior to usage for 1 hour.

After cells were 90 % confluent they were washed once with PBS, trypsinized with 0.25 % trypsin/EDTA (cardiac fibroblasts) or scraped (BMDMs) and neutralized with DMEM. Cells were spun down by centrifugation at 1500 rpm for 5 min and the pellet was re-suspended in fresh DMEM. Cells were counted with the Invitrogen Countess™ automated cell counter using Countess™ cell counting chamber slides (Invitrogen) and Trypan Blue stain 0.4 % (life technologies). At a volume of 100 µl and a number of 1.0x104 – 3.0x104 per well (cardiac fibroblasts) or 1.0x105 per well (BMDMs), cells were seeded in the 96 well tissue culture plate with coated β-tripeptide fibres. Cell- and peptide-free wells with DMEM only were used as blank controls. Laminin-coated wells (2 µg/cm^2) were used as a positive control. Cells were incubated for 2 hours on scaffolds at 37 °C, 5 % CO_2 to allow cell adhesion and cells were then

washed once with PBS and new media was added. Due to the low seeding density, cardiac fibroblasts were allowed to recover overnight in order to reactivate metabolism. Then 5 mg/ml MTT dye (Sigma Aldrich) in PBS was added in a 1:10 dilution to the media and incubated for 4 hours for cardiac fibroblasts or 30 minutes for BMDMs in which viable cells convert the dye to its coloured formazan product. The reaction was terminated by removal of medium with dye and 100 µl DMSO was added to dissolve formazan crystals. The plate was read after 5 min shaking at 120 rpm in a VersaMax micro plate reader (Molecular Devices) at 570 nm and 630 nm as reference. Optical density (OD) was calculated as the difference of absorbance at 570 nm and 630 nm. Cell viability was determined with respect to the control.

For immunofluorescence staining, fibroblasts were grown to subconfluence in 24-well plates on 12 mm glass coverslips (Menzel-Gläser) and then fixed with ice-cold acetone at -20 °C for 5 mins. After washing 3x 10 min with PBS, cells were blocked with 5 % BSA (Albumin from bovine serum (≥98%), Sigma Aldrich) for 30 min and then incubated with a 1:250 dilution of primary antibody "Vimentin (C-20) Sc-7557 goat polyclonal IgG, 200µg/ml" (Santa Cruz Biotech) in antibody diluent (Dako) overnight at 4 °C. The antibody was discarded and cells washed 3x 10 min with PBS before 1 h incubation with secondary antibody "Fluorescein anti-goat polyclonal IgG, FI-5000" (Vector Laboratories) at a 1:500 dilution in antibody diluent. Again, antibody was discarded and cells washed 3x 10min with PBS. Then coverslips with cells were mounted on microscope slides (Menzel-Gläser) using Vectashield mounting medium for fluorescence with DAPI (Vector Laboratories, Inc). Samples were observed in a Nikon Eclipse TE2000-U microscope.

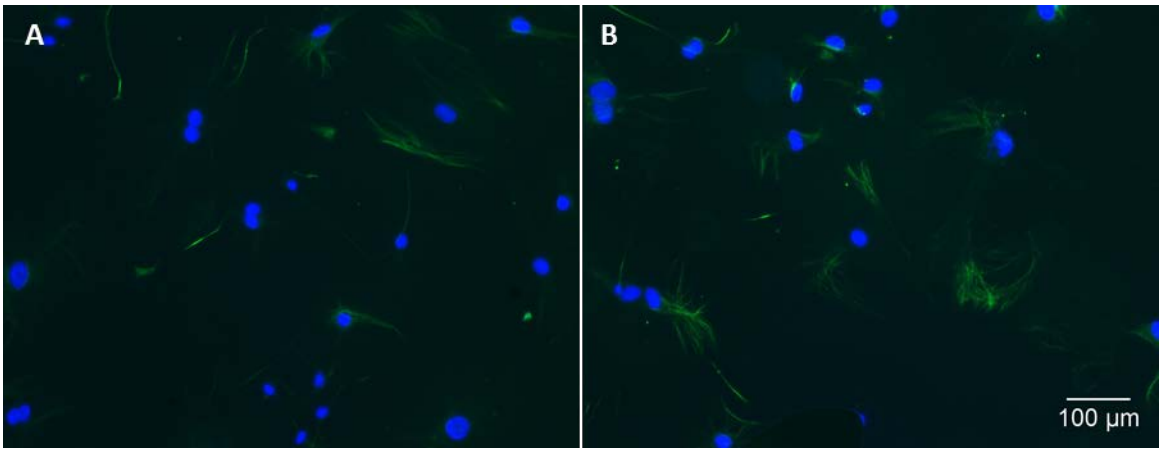

Figure SI-9: Immunofluorescence staining of mouse cardiac fibroblasts grown for 3 days on 12 mm coverslips either on (A) plain glass or (B) on glass coated with 12.5 µg peptide **A** fibres. Cells were then fixed and stained for vimentin (green) and nucleus (blue).

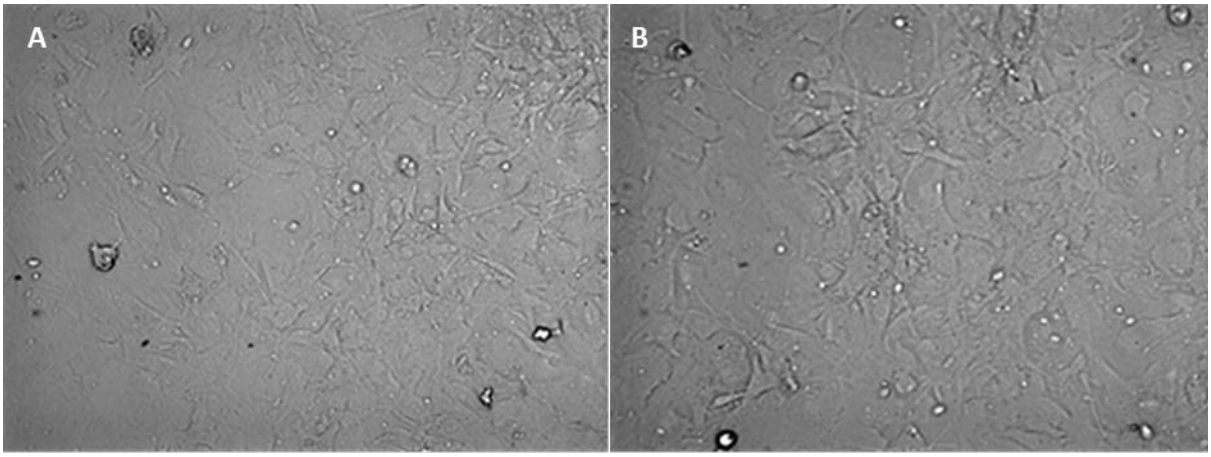

Figure SI-10: Bright field microscopy images of mouse cardiac fibroblasts grown for 20 hours on either a tissue culture plate surface or on a scaffold derived from peptide **A**.

All animal and experimental procedures were approved by the Monash University Animal Ethics Committee (Ethics # SOBSB/PHAR/2010/23).