Supplementary Information (SI) for Lab on a Chip.

A new biofunctionalized and micropatterned PDMS is able to promote stretching induced human myotube maturation. Supplementary Data

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I) Supplementary Figures:

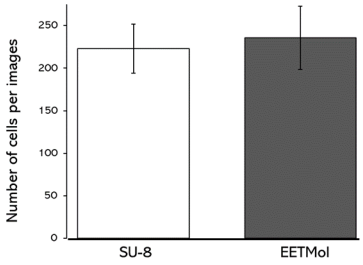


Figure A: In each condition, 50,000 cells were plated in a 35 mm petri dish on day 0. After 3 days, cells were fixed and stained with DAPI for nuclear counting. Ten pictures per condition were analyzed using FIJI software.

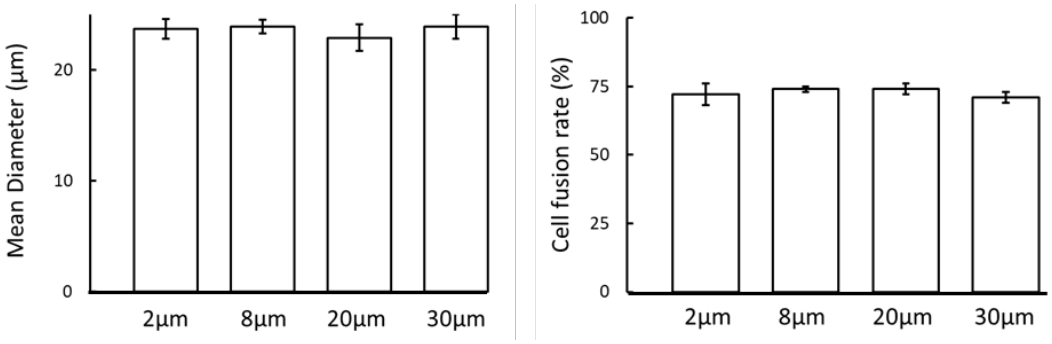


Figure B: Number of cells obtained after 3 days of human primary myoblast culture with photostructured EETMol on a glass substrate, in comparison to SU-8**.**

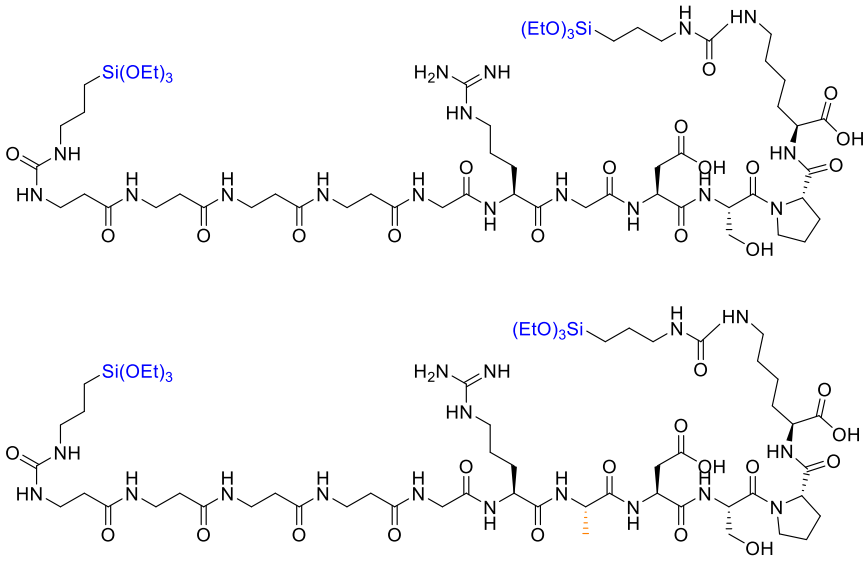


Figure C: Biofunctionalization by peptide-grafting on PDMS. Chemical structures of the hybrid bis-silylated RGD (top) and RAD (bottom) peptides. The triethoxysilane groups (in blue) were introduced at the N-terminus through an (βAla4) spacer and the side chain of a C-terminal lysine residue, by reaction with isocyanatopropyl triethoxysilane. The difference between the two peptide sequences (methyl side-chain) is highlighted in orange.

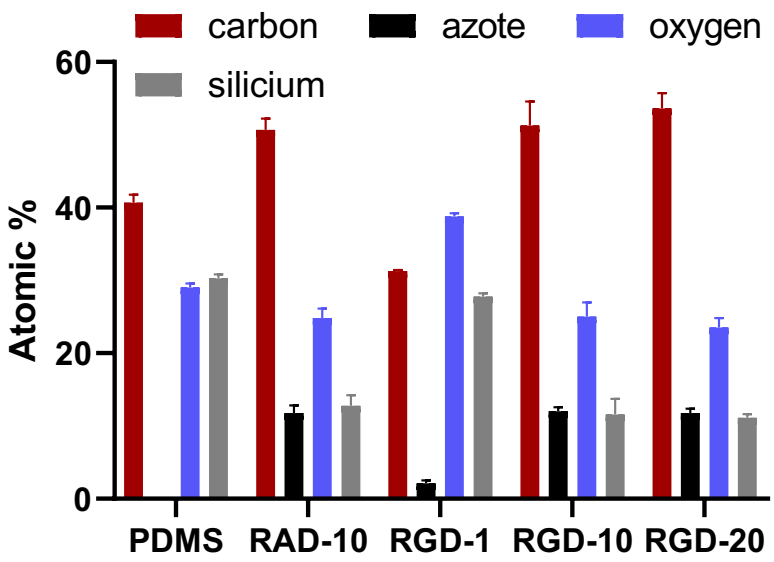


Figure D: Comparative XPS analysis of RGD biofunctionalized PDMS membranes compared to bare PDMS and RAD functionalized membranes. In all five samples, the trio of carbon C1S, silicon S2p, and oxygen O1s characteristic of PDMS is present. Nitrogen N1S is present in all the samples functionalized with peptides. When the concentration exceeds 1 mg/mL, the presence of nitrogen increases, while that of silicon decreases, indicating the formation of a layer.

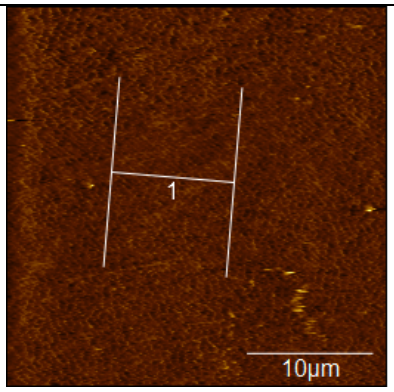
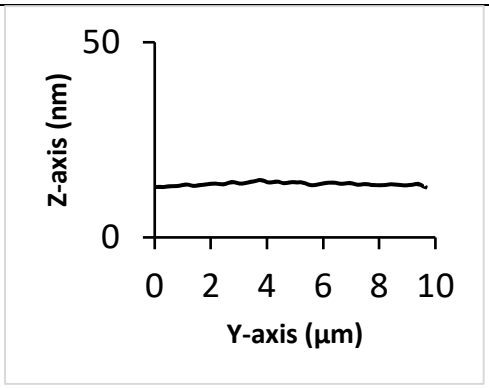
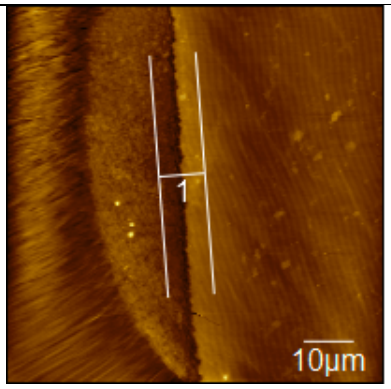
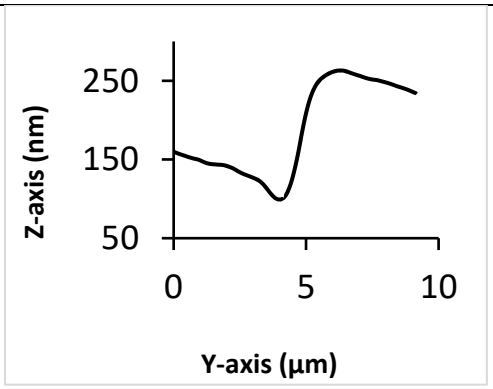
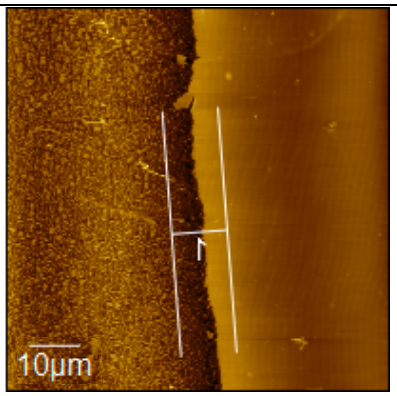
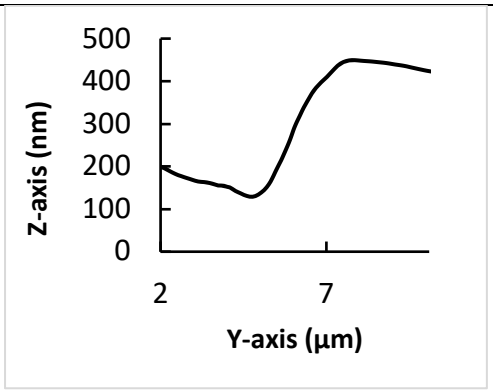
Sample	Image	Profile	Coating height
Bare PDMS			**0 nm**
Silylated RGD-functionalized (10 mg/mL)			**150 nm**
Silylated RGD-functionalized (20 mg/mL)			**380 nm**

Figure E: AFM analysis results: The surface morphology of the biofunctionalized PDMS membranes was observed using an Atomic Force Microscope (AFM) Step 700 (Anton Paar GmbH, Austria) operating in air under ambient conditions. Thickness measurements of the RGD peptide coating on PDMS were carried out in tapping mode on a scan area fixed at 20 µm × 20 µm.

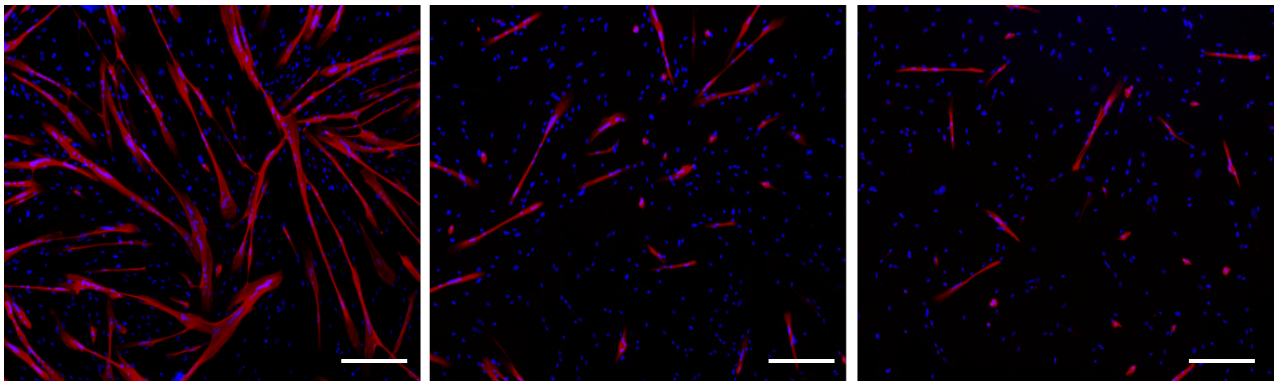


Figure F : Myogenic progenitor cells were seeded and cultured for 3 days of proliferation and 3 days of differentiation on a silylated RGD-functionalized membrane (left, 10 mg/mL), a silylated RAD-functionalized membrane (middle, 10 mg/mL), and bare PDMS (right). Myotubes are stained in red with Troponin T as detailed in the materials and methods section. Scale bar: 200 µm.

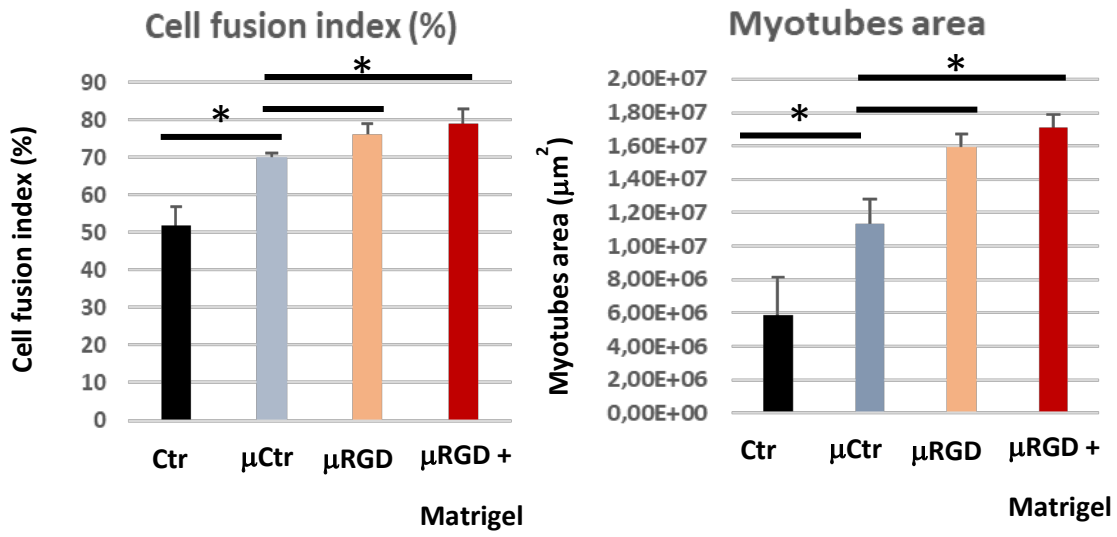


Figure G Effect different coating condition on Myotube differenciation:

Left panel: Cell fusion rate; Right panel Myotube area; Ctr: bare PDMS; µCTR : microstructured bare PDMS; µRGD: microstructured PDMS and functionalized with RGD; µRGD + Matrigel: microstructured PDMS functionalized with RGD and Matrigel; $p < 0.05$ (*).

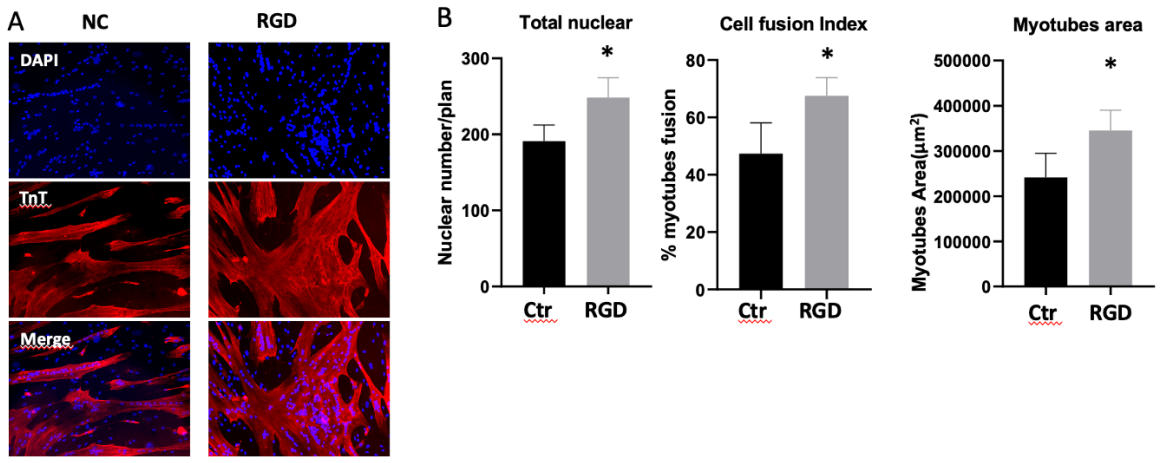


Figure H: Stability of RGD coating with time.

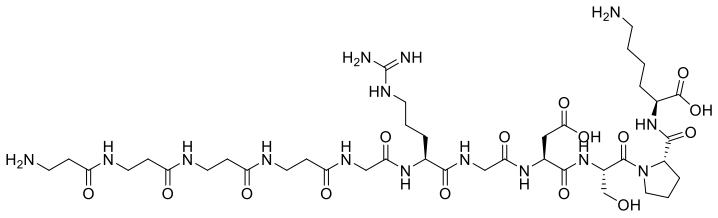
Panel A) Confocal imaging of myotubes stained in red with Troponin T (TnT) and nuclei stained with DAPI in blue; Panel B) 3 Parameters of cell attachment and myotubes differentiation: Nuclear Number/plan, cell fusion index and Myotube area; Ctr: bare PDMS; RGD: 18 month old PDMS coated with RGD peptides; $p < 0.05$ (*).

Supplementary Materials and methods

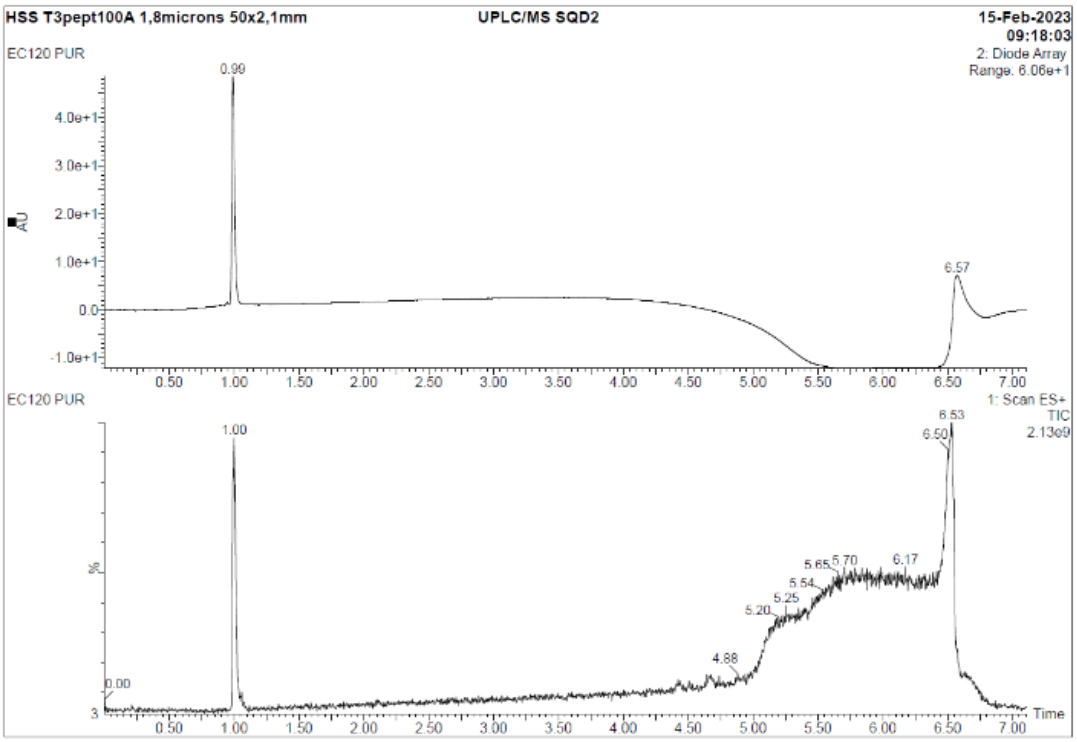
Peptides synthesis:

All solvents and reagents were used as supplied. Anhydrous DMF was used and purchased from Acros. NMR solvents were obtained from Eurisotop. Isocyanatopropyl triethoxysilane was obtained from TCI Europe. All amino acid derivatives and HATU (1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate were purchased from Iris Biotech GmbH (Marktredwitz, Germany). 2.2-Cl-Chlorotrityl resin and Fmoc-L-Lys(Boc)-wang (loading 0.66 mmol/g) were acquired from Iris Biotech GmbH (Marktredwitz, Germany). Solvents were purchased from Carlo Erba reagents (Val de Reuil, France). Trifluoroacetic acid, 99% extra pure, was obtained from Acros Organics (New Jersey, USA). Other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA). Fmoc aminoacid were dissolved in DMF at 0.5 M concentration.

Synthesis of RGD peptide: H-(βAla)$_4$-Gly-Arg-Gly-Asp-Ser-Pro-Lys-OH, 3 TFA



Synthesis by SPPS was made on a multipep1 CEM synthesizer using the Fmoc-L-Lys(boc)-wang (loading 0.66 mmol/g, 5x151mg = 5x0.1mmol) resin in DMF. Coupling reactions were performed using a mixture of a Fmoc amino acid solution (0.5M in DMF, 4eq) / DIEA solution (1.2M in NMP, 8eq) / HATU solution (0.5M in DMF, 4eq) for 20 minutes twice following by a capping step (5 minutes with 2mL of an acetic anhydride/DMF solution 5/95 v/v). The Fmoc removal steps were realized using a piperidine/DMF 20/80 v/v solution (5 mL) for 1 minute and performed twice. All washings were done with DMF twice (5 mL) after coupling steps and thrice (5 mL) after deprotection steps. Just before the cleavage, the resin was washed with DCM twice (2 mL). The peptide was cleaved from the resin with a mixture of TFA/TIS/H2O (94/3/3, v/v/v) for 30 minutes, concentrated under reduced pressure, recovered by precipitation in diethyl ether, then taken up in ACN/H2O 50/50 v/v mixture and freeze-dried. The crude peptide was solubilized in H_2O with 1‰ TFA and purified in two injections by RP-preparative HPLC. The purification was performed on the Gilson system. Eluents were H_2O 1‰ TFA (A) and acetonitrile 1‰ TFA (B). The purification gradient started with 5 minutes at 5% of B, then increased from 5 to 20% of B in 15 min. Collected fractions were concentrated and freeze-dried to yield 431.9 mg of the RGD peptide as a TFA salt (white powder) with 86 % yield.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EC120 PUR
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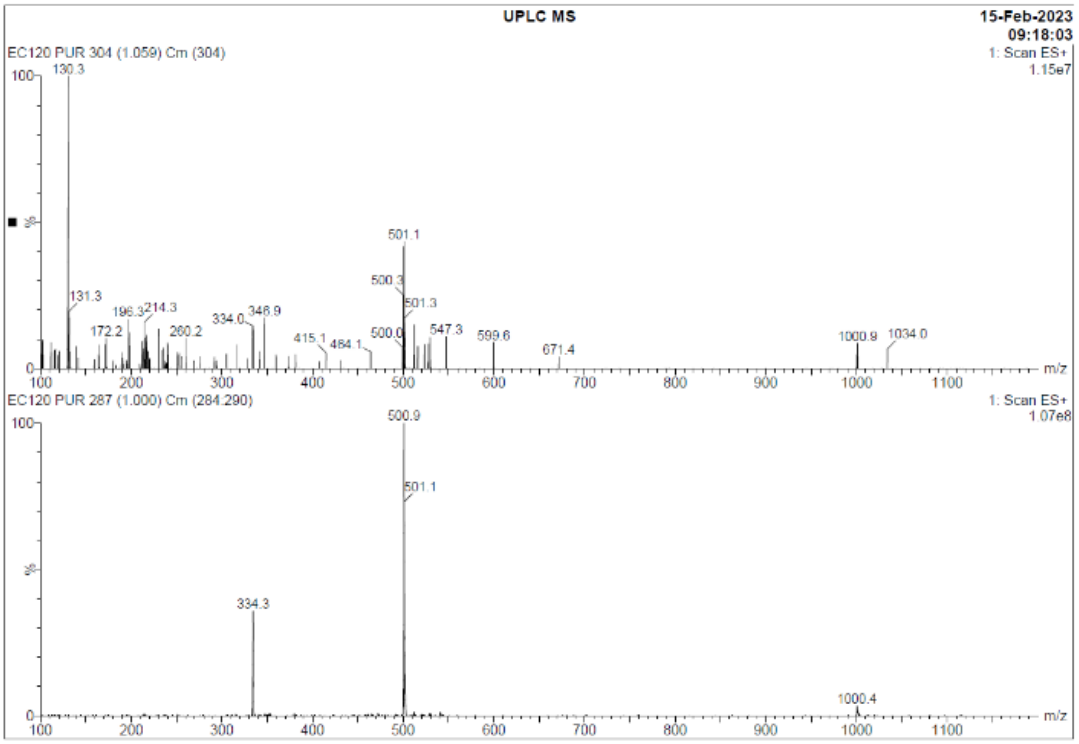
Range: 6.06e+1
0.99
6.57
AU
EC120 PUR
1: Scan ES+
TIC
2.13e9
1.00
6.53
6.50
5.65
5.70
6.17
5.54
5.25
5.20
4.88
0.00
%
Time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15-Feb-2023
09:18:03
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500.3
501.3
131.3
196.3
214.3
346.9
172.2
260.2
334.0
415.1
484.1
500.0
547.3
599.6
671.4
1000.9
1034.0
m/z
EC120 PUR 287 (1.000) Cm (284.290)
1: Scan ES+
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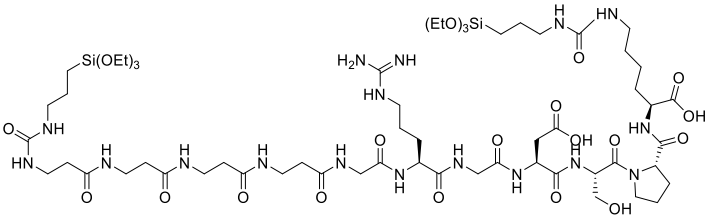
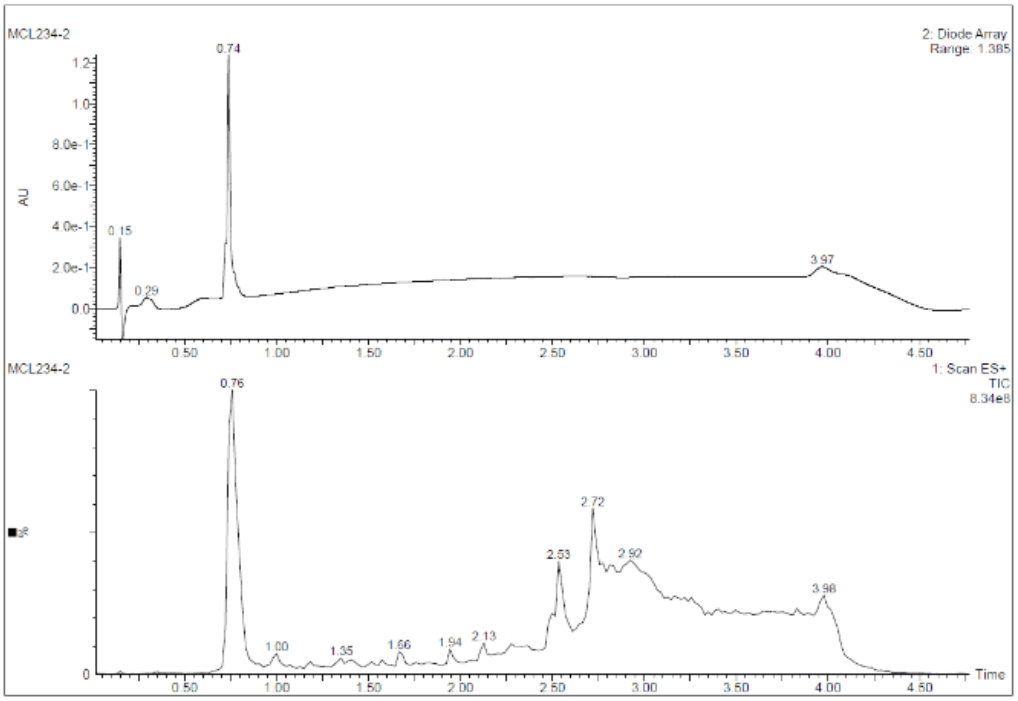
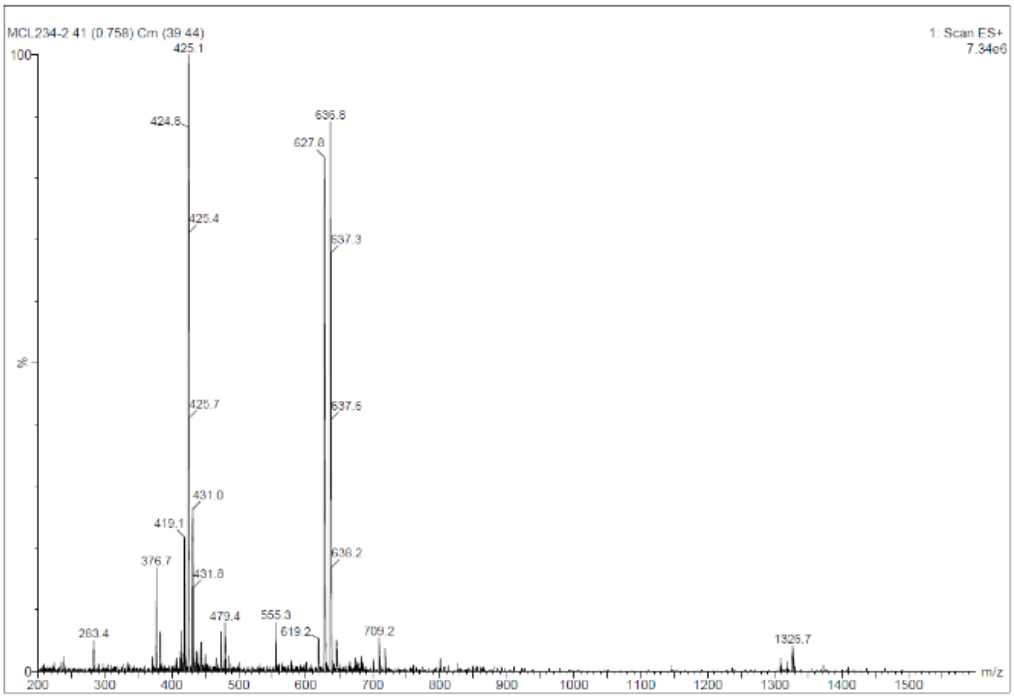
501.1
334.3
1000.4
%

Synthesis of the bis-silylated RGD peptide $(EtO)_3Si$-$(CH_2)_3NHCO$-$(\beta Ala)_4$-Gly-Arg-Gly-Asp-Ser-Pro-Lys($(EtO)_3Si$-$(CH_2)_3NHCO$)-OH by bis-silylation of the RGD peptide.

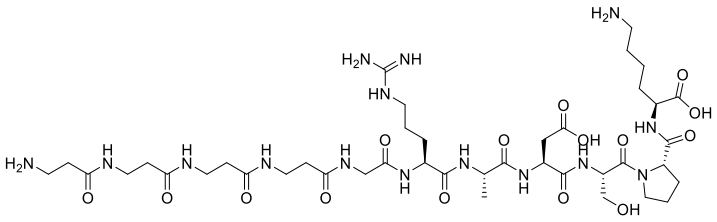


The RGD peptide (0.300 g, 0.3 mmol, 1eq) was solubilized in anhydrous DMF (8 mL). DIEA (522 µL, 3 mmol, 10eq) and ICPTES (284 µL, 0.6 mmol, 4eq) were added under stirring and inert atmosphere (argon). The solution was stirred at RT for 4h. The hybrid peptide was precipitated by addition of diethyl ether and the obtained white solid was washed 3 times with diethyl ether and vacuum dried. 340 mg of the bis-silylated RGD peptide were obtained (86% yield). This bis-silylated peptide was kept under inert atmosphere at -4°C and used in the 15 days following its preparation to avoid unwanted premature condensation.



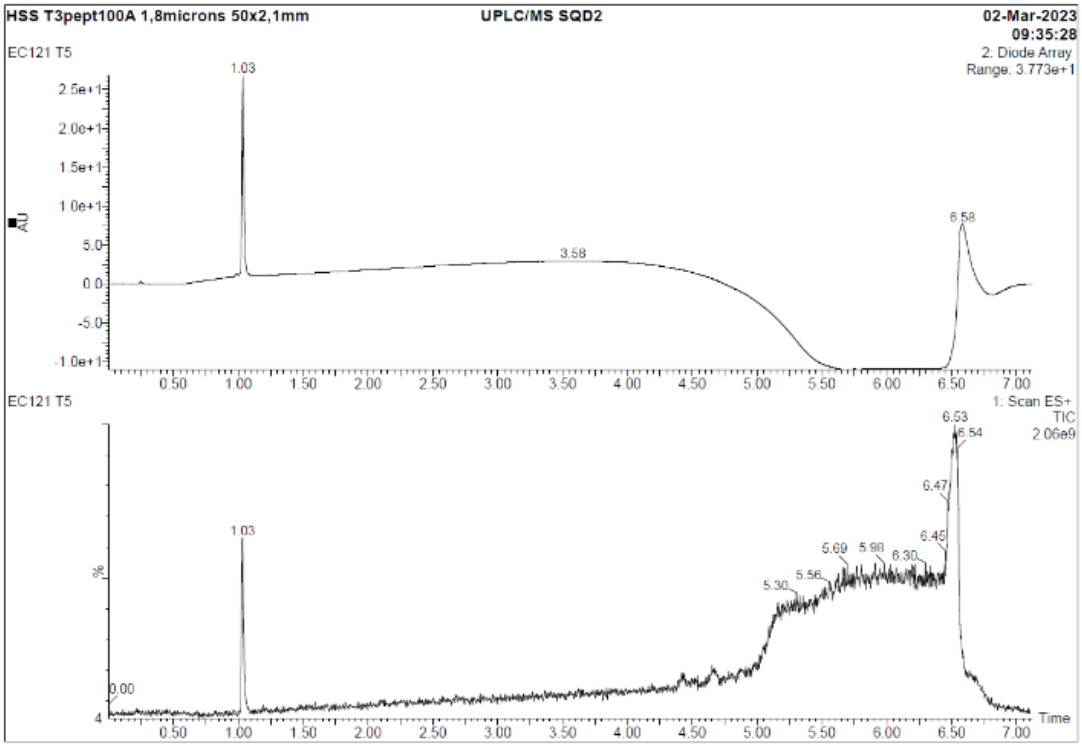


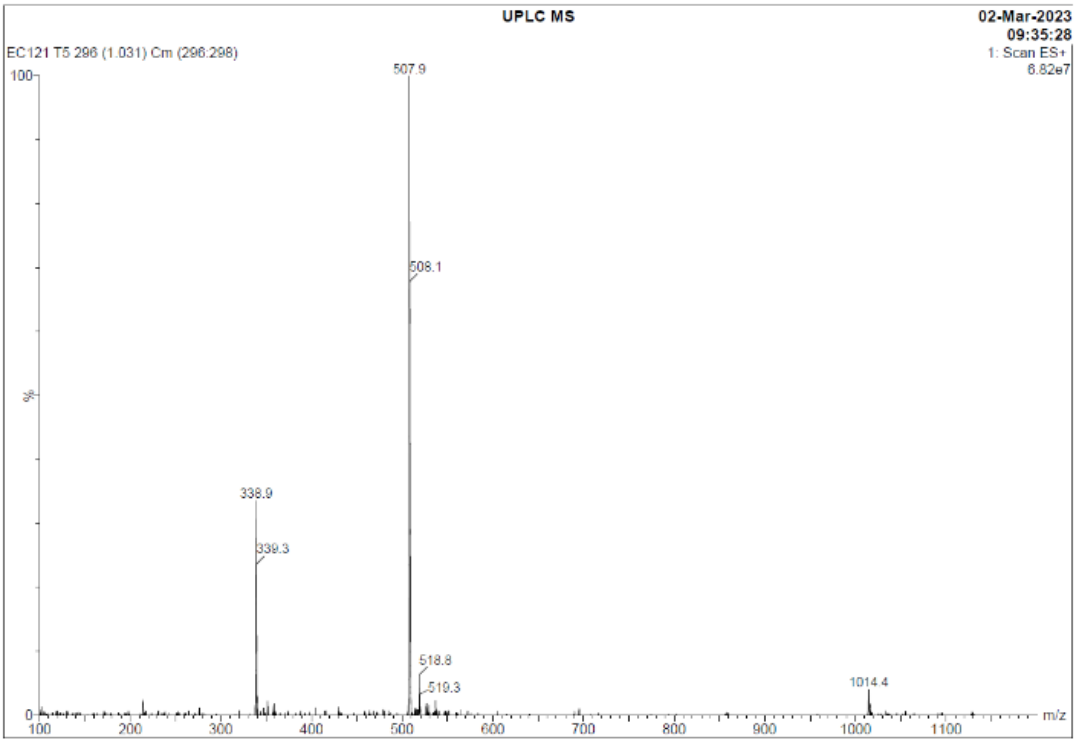
Synthesis of the RAD peptide: H-(βAla)4-Gly-Arg-Ala-Asp-Ser-Pro-Lys-OH, 3 TFA



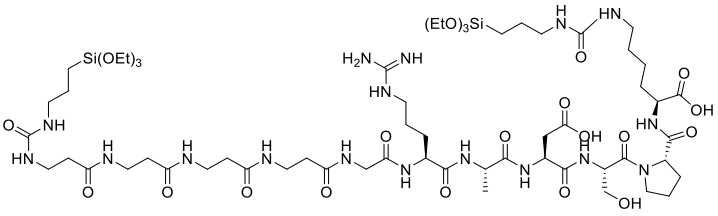
Synthesis by SPPS was made on a multipep1 CEM synthesizer using the Fmoc-L-Lys(boc)-wang (loading 0.66 mmol/g, 151mg, 0.1mmol) resin in DMF. Coupling reactions were performed using a mixture of a Fmoc amino acid solution (0.5M in DMF, 4eq) / DIEA solution (1.2M in NMP, 8eq) / HATU solution (0.5M in DMF, 4eq) for 20 minutes twice following by a capping step (5 minutes with 2mL of an acetic anhydride/DMF solution 5/95 v/v). The Fmoc removal steps were realized using a piperidine/DMF 20/80 v/v solution (2 mL) for 3 minute and performed twice. All washings were done with DMF twice (5 mL) after coupling steps and thrice (5 mL) after deprotection steps. Just before the cleavage, the resin was washed with DCM twice (5 mL). The peptide was cleaved from the resin with a mixture of TFA/TIS/H_2O (94/3/3, v/v/v) for 30 minutes, concentrated under reduced pressure, recovered by precipitation in diethyl ether, then taken up in ACN/H_2O 50/50 v/v mixture and freeze-dried. The crude peptide was solubilized in H_2O with 1‰ TFA and purified in two injections by RP-preparative HPLC. The purification was performed on the Gilson system. Eluents were H_2O 1‰ TFA (A) and acetonitrile 1‰ TFA (B). The purification gradient started with 5 minutes at 0% of B, then increased from 0 to 5% of B

in 3 min, then increased from 5% to 20% in 15 minutes. Collected fractions were concentrated and freeze-dried to yield 77 mg of the pure RAD peptide as a TFA salt (white powder) with 76 % yield.

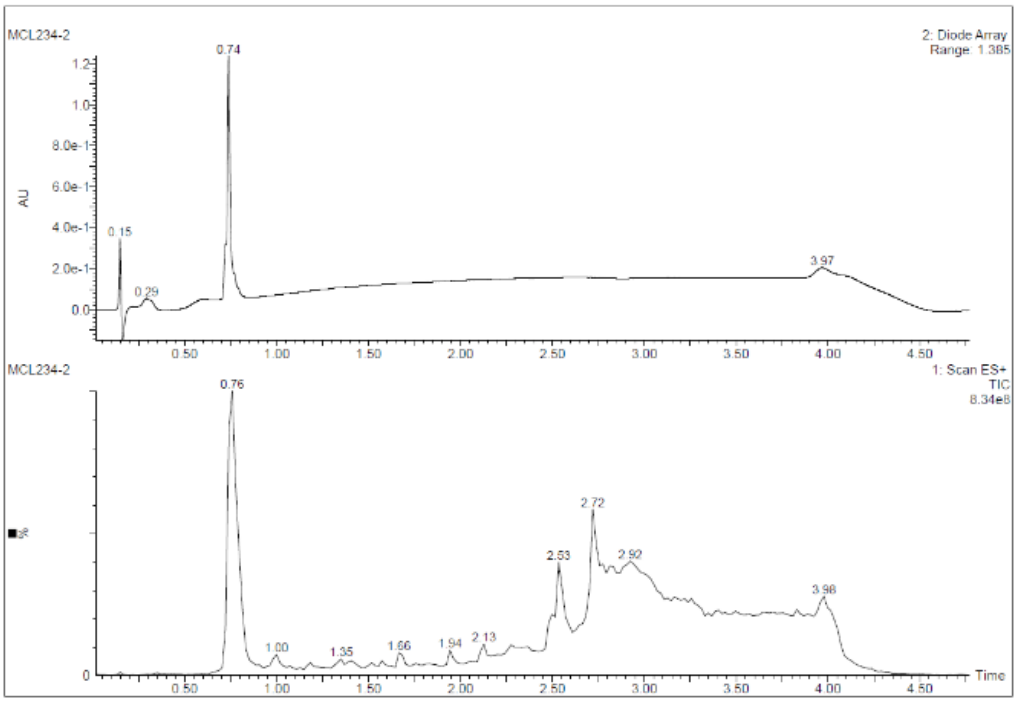


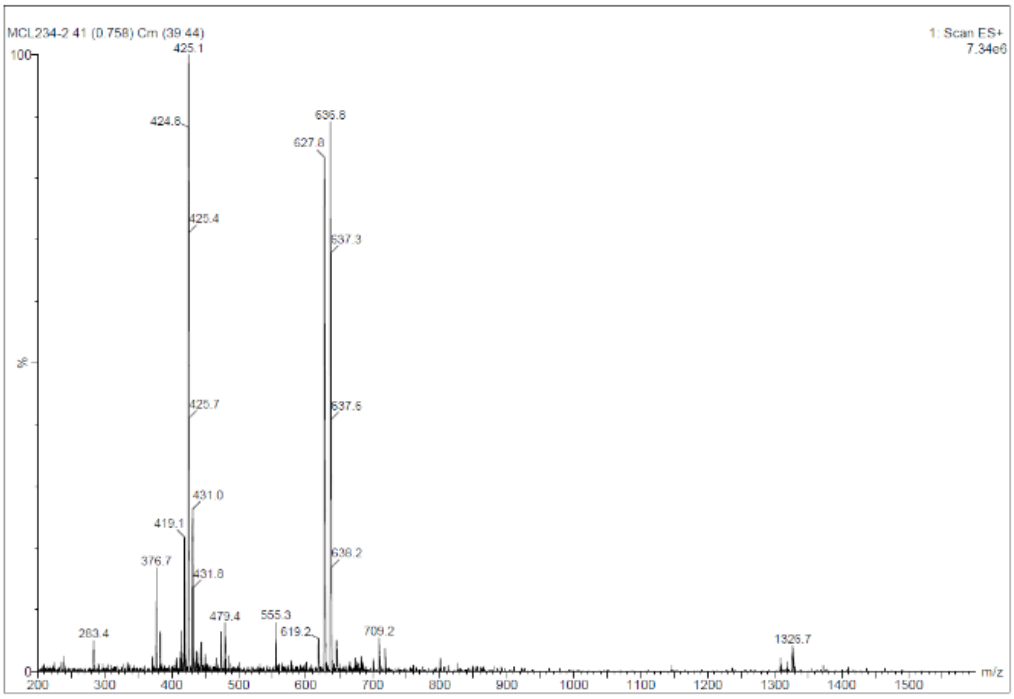


Synthesis of the bis-silylated RAD peptide $(EtO)_3Si$-$(CH_2)_3NHCO$-$(\beta Ala)_4$-Gly-Arg-Ala-Asp-Ser-Pro-Lys($(EtO)_3Si$-$(CH_2)_3NHCO$)-OH 1-Si by bis-silylation of RAD peptide .



The RAD peptide (0.077 g, 0.076 mmol, 1eq) was solubilized in anhydrous DMF (4 mL). DIEA (132 µL, 0.74 mmol, 10eq) and ICPTES (72 µL, 0.304 mmol, 4eq) were added under stirring and inert atmosphere (argon). The solution was stirred at RT for 4h. The hybrid peptide was precipitated by addition of diethyl ether and the obtained white solid was washed 3 times with diethyl ether and vacuum dried. 97 mg of bis-silylated RAD peptide were obtained (96% yield). This bis-silylated peptide was kept under inert atmosphere at -4°C and used in the 15 days following its preparation to avoid unwanted premature condensation.

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%
0
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424.8
636.8
627.8
425.4
637.3
425.7
637.6
431.0
419.1
638.2
376.7
431.8
479.4
555.3
619.2
709.2
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400
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600
700
800
900
1000
1100
1200
1300
1400
1500
m/z

Primers used:

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	reverse	**GAGGAGACCACCAAGATGCC**