SUPPORTING INFORMATION

Nanostructured interfacial self-assembled peptide-polymer membranes for enhanced mineralization and cell adhesion

Sofia C. Ribeiro,[a,b] Elham Radvar,[c,d] Yejiao Shi, [c,d]João Borges,[e] Rogério P. Pirraco,[a,b] Isabel B. Leonor,[a,b] João F. Mano,[e] Rui L. Reis,[a,b] Álvaro Mata[c,d] and Helena S. Azevedo*[,a,b,c,d]

[a]3B's Research Group – Biomaterials, Biodegradables and Biomimetics, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, University of Minho, AvePark, 4806-909 Taipas, Guimarães, Portugal;

[b]ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

[c]School of Engineering and Materials Science, Queen Mary University of London, London E1 4NS United Kingdom

[d]Institute of Bioengineering, Queen Mary, University of London, London E1 4NS, United Kingdom

[e]Department of Chemistry, CICECO – Aveiro Institute of Materials, University of Aveiro, 3810-193 Aveiro, Portugal

*Corresponding author
E-mail: h.azevedo@qmul.ac.uk
Tel: +44 (0)20 7882 5502

Table of contents

S1. Molecular and structural information of the MDPs used for fabricating the self-assembling membranes

Table S1 Molecular information of MDPs used in this study

MDP	Sequence	Chemical structure	Expected mass
$K_2(QL)_6K_2$	$CH_3CONH\text{-}K_2(QL)_6K_2\text{-}CONH_2$	$C_{92}H_{167}N_{27}O_{23}$	2019.51
$K_2(SV)_6K_2$	$CH_3CONH\text{-}K_2(SV)_6K_2\text{-}CONH_2$	$C_{74}H_{137}N_{21}O_{23}$	1689.03
$K_3(QL)_6E_2$	$CH_3CONH\text{-}K_3(QL)_6E_2\text{-}COOH$	$C_{96}H_{168}N_{26}O_{29}$	2150.55
$K_3(QL)_6E_2RGDS$	$CH_3CONH\text{-}K_3(QL)_6E_2RGDS\text{-}CONH_2$	$C_{111}H_{194}N_{34}O_{35}$	2564.98
$K_3(QL)_6K_2YGFGG$	$CH_3CONH\text{-}K_3(QL)_6K_2YGFGG\text{-}CONH_2$	$C_{120}H_{196}N_{32}O_{34}$	2631.08

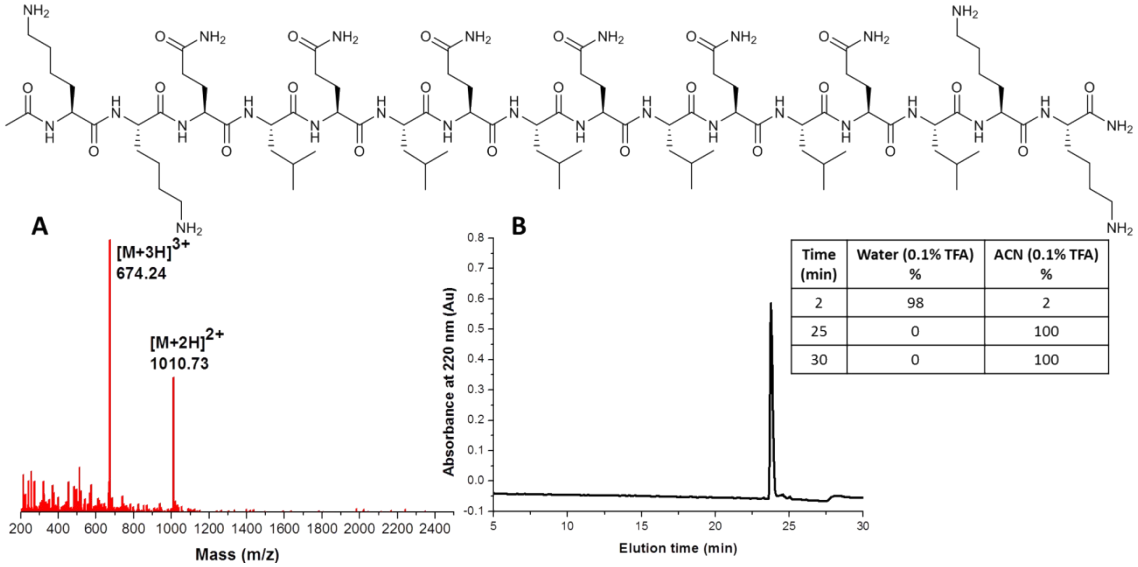


Fig. S1 (A) ESI-MS and (B) RP-HPLC trace (with corresponding mobile phase gradient) of purified $K_2(QL)_6K_2$ (98% purity).

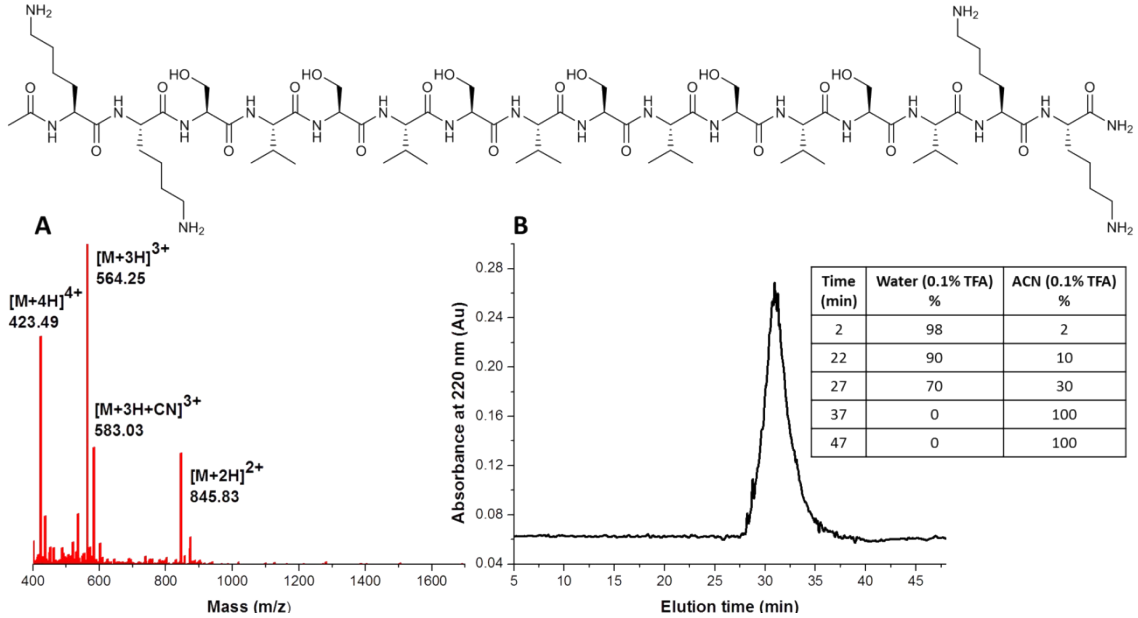


Fig. S2 (A) ESI-MS and (B) RP-HPLC trace (with corresponding mobile phase gradient) of purified $K_2(SV)_6K_2$ (95% purity).

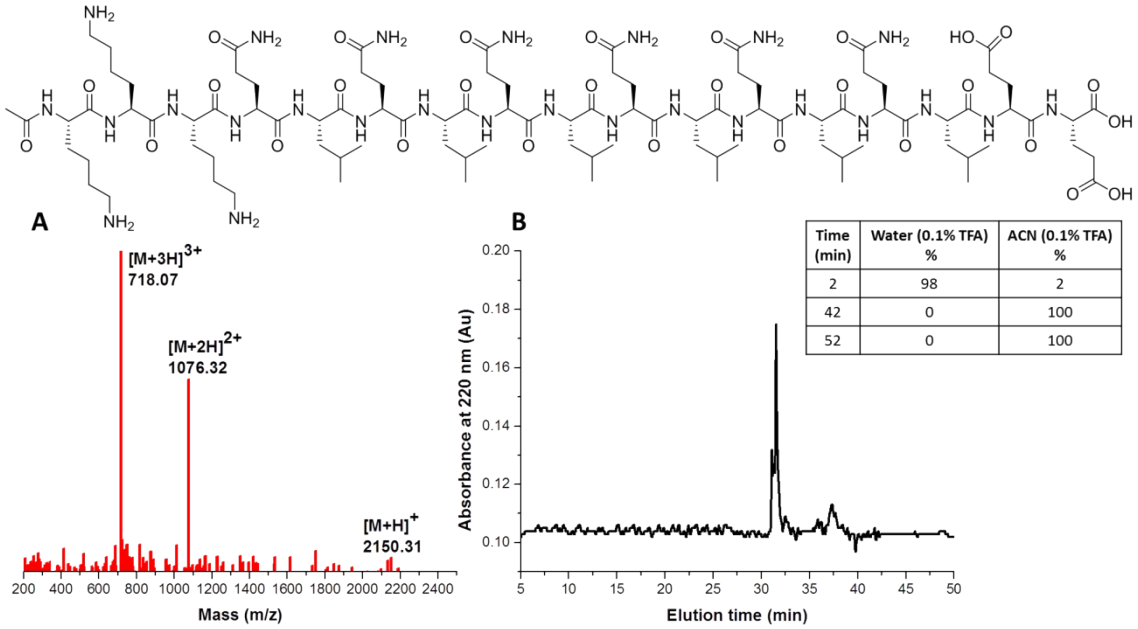


Fig. S3 (A) ESI-MS and (B) RP-HPLC trace (with corresponding mobile phase gradient) of purified $K_3(QL)_6E_2$ (70% purity).

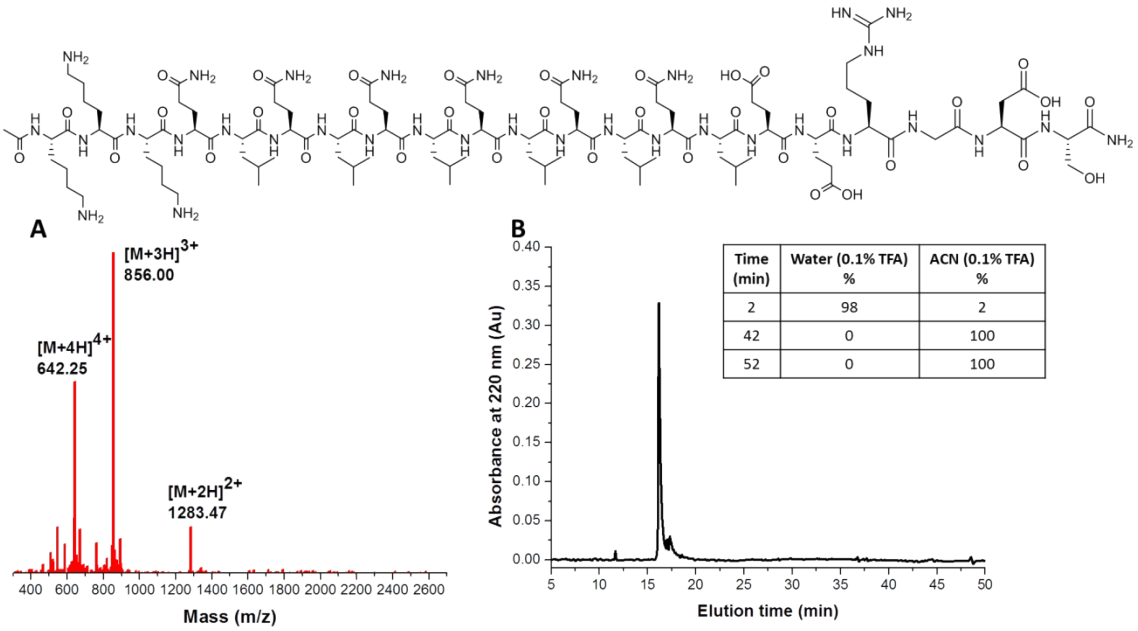
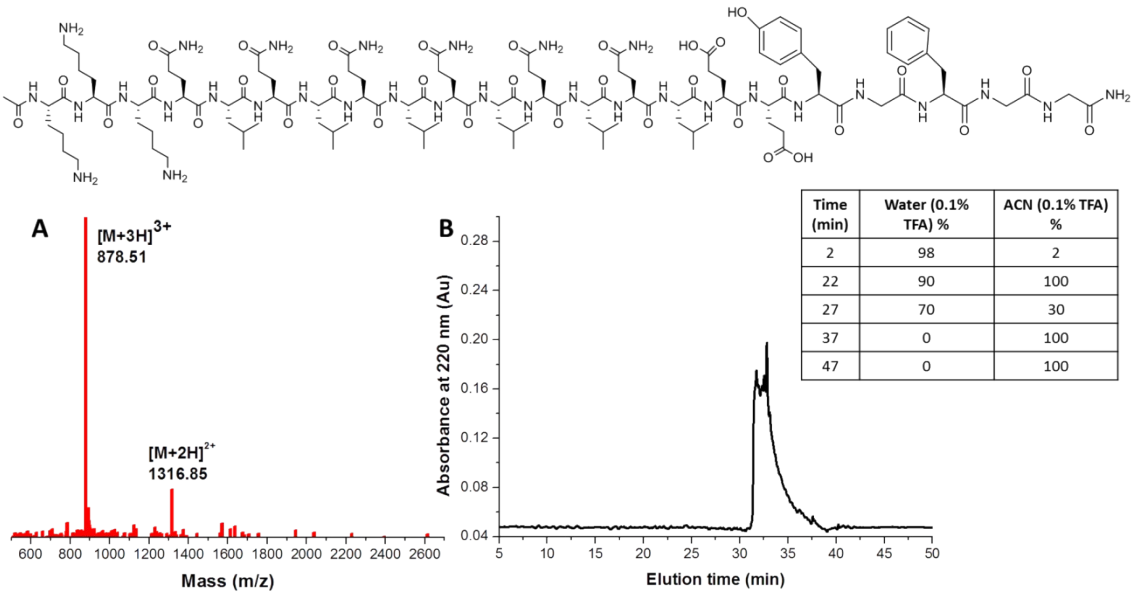


Fig. S4 (A) ESI-MS and (B) RP-HPLC trace (with corresponding mobile phase gradient) of purified $K_3(QL)_6E_2RGDS$ (95% purity).



Time (min)	Water (0.1% TFA) %	ACN (0.1% TFA) %
2	98	2
22	90	100
27	70	30
37	0	100
47	0	100

Fig. S5 (A) ESI-MS and (B) RP-HPLC trace (with corresponding mobile phase gradient) of purified $K_3(QL)_6E_2YGFGG$.

Table S2 Zeta potential of MDP solutions (0.1 wt%).

pH	$K_2(QL)_6K_2$	$K_2(SV)_6K_2$	$K_3(QL)_6E_2$	$K_3(QL)_6E_2RGDS$	$K_3(QL)_6E_2YGFGG$
3	82.33 ± 2.1	55.03 ± 2.1	45.83 ± 0.8	55.43 ± 0.4	35.97 ± 0.3
7	65.70 ± 2.8	48.10 ± 1.3	-1.48 ± 1.3	44.57 ± 0.3	10.57 ± 0.1
9	23.42 ± 2.3	21.33 ± 1.3	-34.37 ± 0.5	5.7 ± 0.1	-18.67 ± 0.2

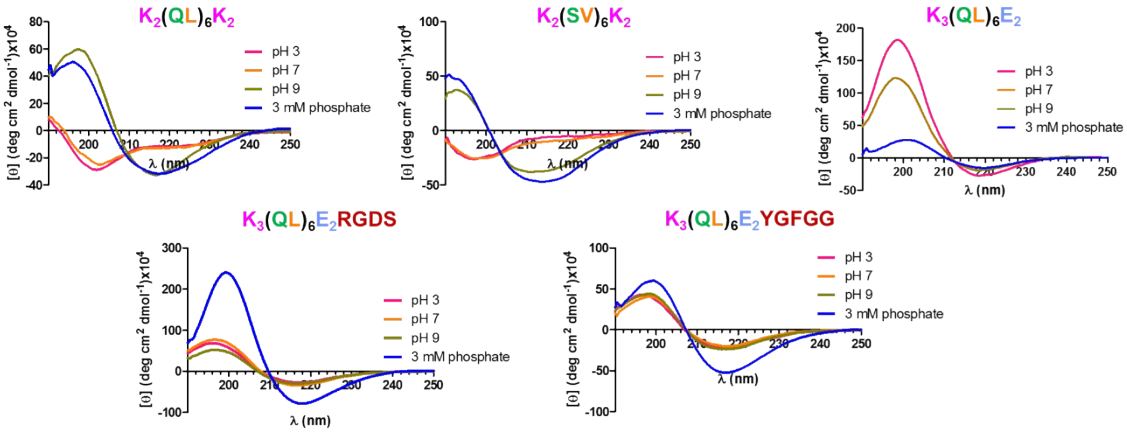
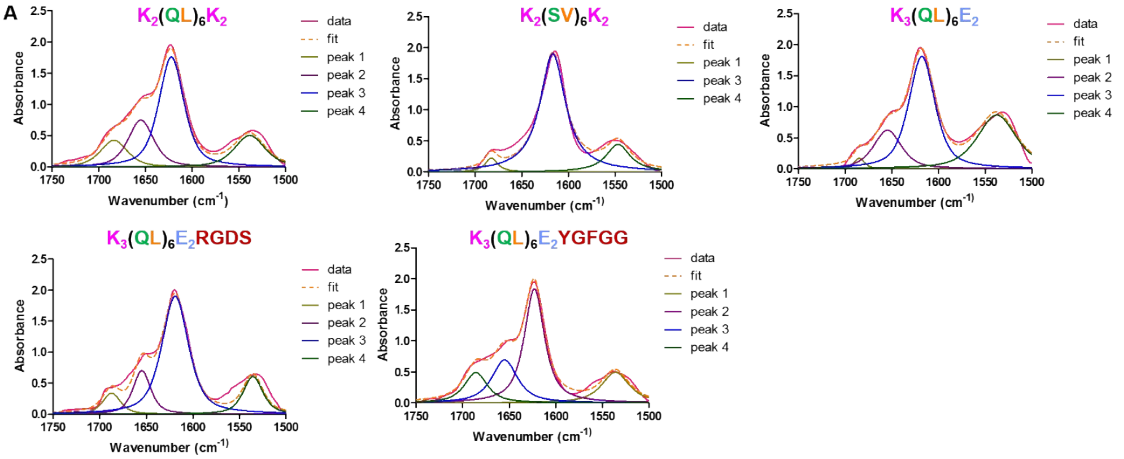


Fig. S6 CD spectra of MDPs. Peptide samples were prepared at 0.011 mM in water (pH 3, 7 and 9) or phosphate solution (pH 7).

A



B

peak	Meaning	estimated position (cm^{-1})	position observed (cm^{-1})				
			$K_2(QL)_6K_2$	$K_2(SV)_6K_2$	$K_3(QL)_6E_2$	$K_3(QL)_6E_2RGDS$	$K_3(QL)_6E_2YGFGG$
1	Amide I⊥, anti-parallel β-sheet	1695	1684	1683	1685	1687	1686
2	Glutamine side chain	1654	1655	-	1655	1655	1655
3	Amide $I_{\parallel}$ β-sheet	1630-1610	1622	1616	1618	1619	1623
4	Amide II	1547-1520	1536	1546	1537	1535	1536

Fig. S7 (A) Deconvoluted ATR-FTIR of MDPs. (B) Estimated and observed wave numbers for deconvoluted peaks.

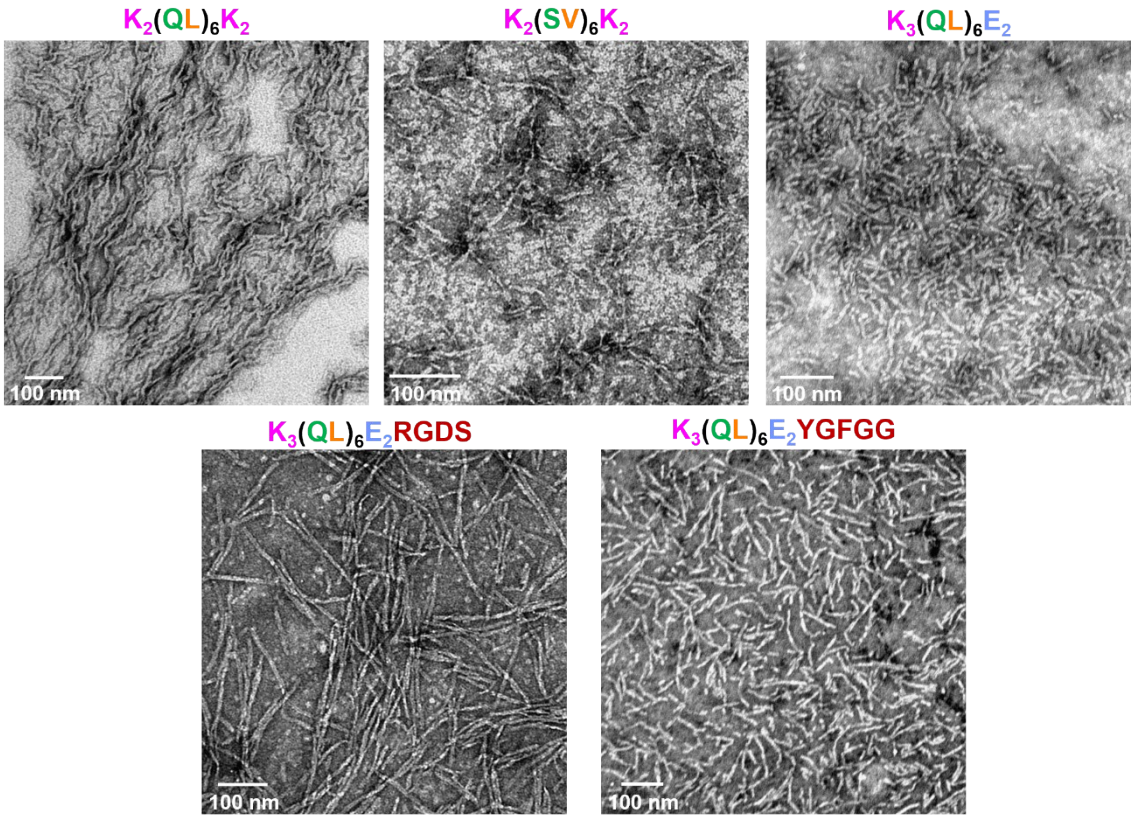


Fig. S8 TEM images of MDPs at 0.01 wt% dissolved in 10 mM phosphate buffer at pH 11.

S2. Scanning electron microscopy images of HA-MDP membranes

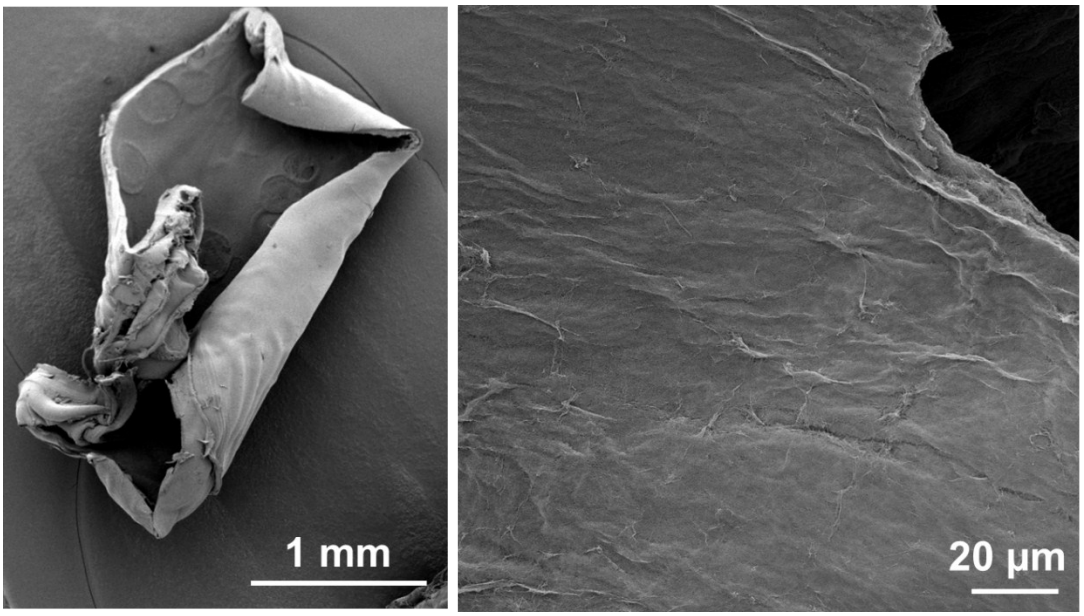


Fig. S9 Representative SEM images of the membranes overall structure showing macro (left) and microscopic (right) details.

S3. Flow cytometry analysis of PDCs

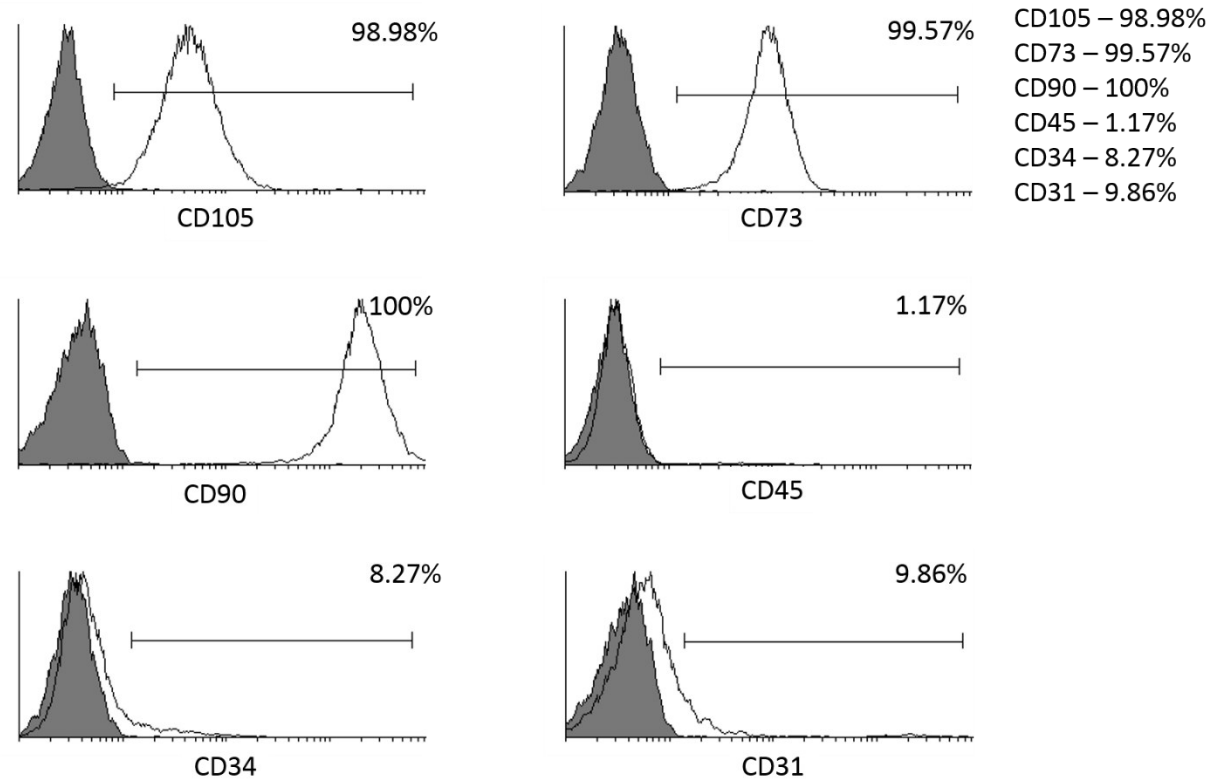


Fig. S10 Flow cytometry analysis of CD105, CD73, CD90, CD45, CD34 and CD31 expression on PDCs showing high expression of specific MSC markers (CD105, CD73, CD90).

S4. Analysis of density and morphology of cells cultured on MDP-coated coverslips and MDP-HA membranes

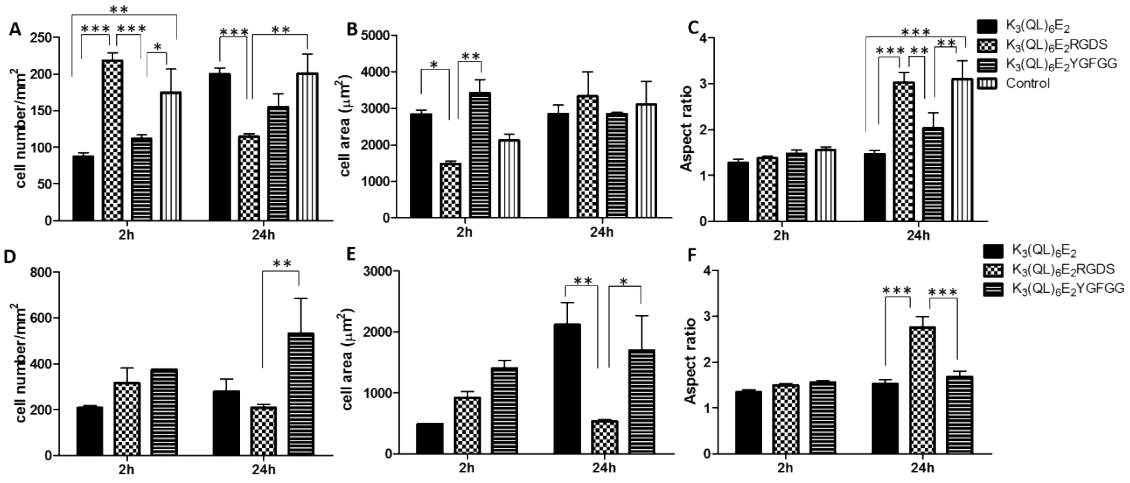


Fig. S11 Average cell density, cell area and aspect ratio of PDCs cultured on uncoated (control) coverslips and coated with MDPs (A, B, C) and on HA-MDP membranes (D, E, F) under serum-free conditions at 2 and 24 hours. ***p<0.001; **p<0.01; *p<0.05), error bars represent standard deviation.

S5. Cell proliferation on HA-MDP membranes

To determine the proliferation potential of PDCs seeded on self-assembled HA-MDP membranes, a similar protocol was used as described in section 2.4.2 (PDC seeding and culture on MDP-coated coverslips and HA-MDP membranes), with the alteration that after 14 hours of culture, the medium was replaced with DMEM containing 10% fetal bovine serum (FBS) to allow cell proliferation for 7 and 14 days. PDCs cultured directly onto the well plate under the same conditions was used as positive control. Cell numbers were estimated by DNA quantification and the morphology of the cells was analysed by SEM. Samples were prepared and imaged as described in 2.4.4 and 2.3.1.

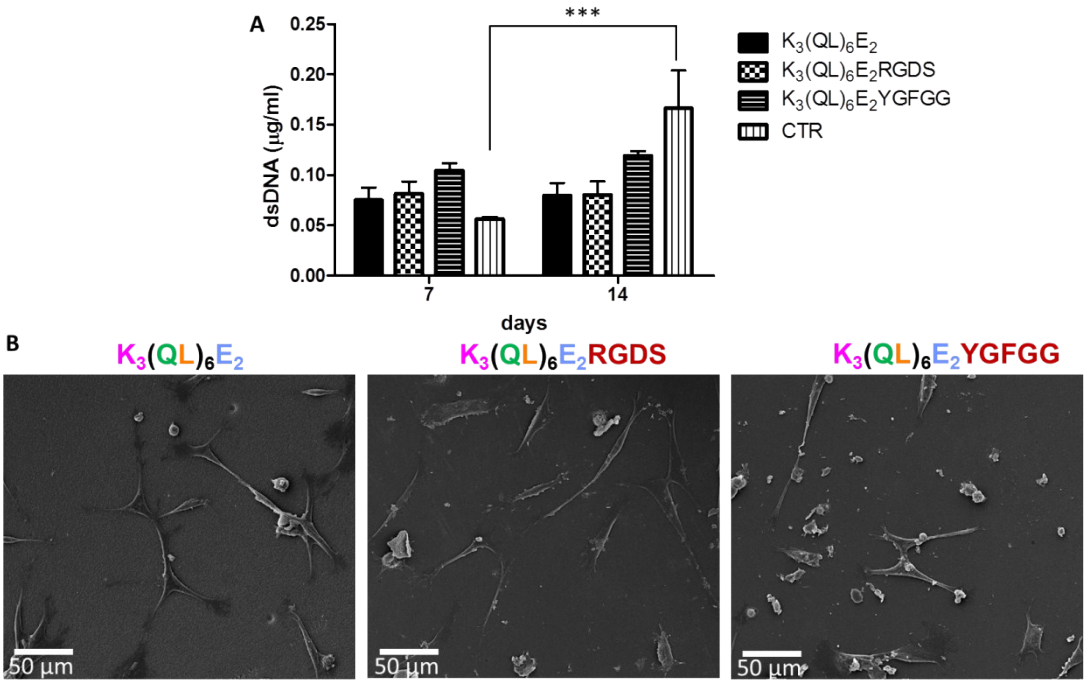


Fig. S12 Cell proliferation assay on HA-MDP membranes with serum supplementation. (A) dsDNA quantification (***p<0.001), error bars represent standard deviation; (B) SEM images showing PDCs on the surface (peptide side) of self-assembled HA-MDP membranes after 14 days of culture.