Electronic Supplementary Material (ESI) for Biomaterials Science

Supplementary Information

Hyaluronan and self-assembling peptides as building blocks to reconstruct the extracellular environment in skin tissue

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Peptide mass and purity

All peptides were synthesized, characterized and purified successfully. ESI and MALDI-MS were used to characterize the mass of the synthesized peptides (Fig. S1-S4, A).

The expected mass for $C_{16}V_3A_3K_3$ ($C_{58}H_{111}N_{13}O_{10}$) was 1150.58, three main peaks were found by ESI-MS, corresponding to $[M+H]^+$ m/z = 1151.30, $[M+Na]^+$ m/z= 1173.25 and $[M+2H]^{2+}$ m/z = 576.48 (Fig. S1A).
The expected mass for $C_{16}V_3A_3K_3RGDS$ ($C_{73}H_{136}N_{20}O_{17}$) was 1565.98, two mains peaks were found, corresponding to $[M+2H]^{2+}$ m/z = 784.09 and $[M+3H]^{3+}$ m/z = 523.39 (Fig. S2A). For $C_{16}V_3A_3K_3DGSR$ ($C_{73}H_{136}N_{20}O_{17}$) the expected mass was 1566.01, two mains peaks were found, corresponding to $[M+2H]^{2+}$ m/z = 784.03 and $[M+3H]^{3+}$ m/z = 523.41 (Fig. S3A).
The expected mass for $C_{16}V_3A_3K_3K_{rhod}RGDS$ was 2108.27, two main peaks were found by MALDI-MS, m/z = 2106.54 and m/z = 1694.34, corresponding to the labeled and unlabeled peptide, respectively (Fig. S4A).

Analytical HPLC of the collected fractions showed a single peak after purification for all the PAs (Fig. S1-S4, B)

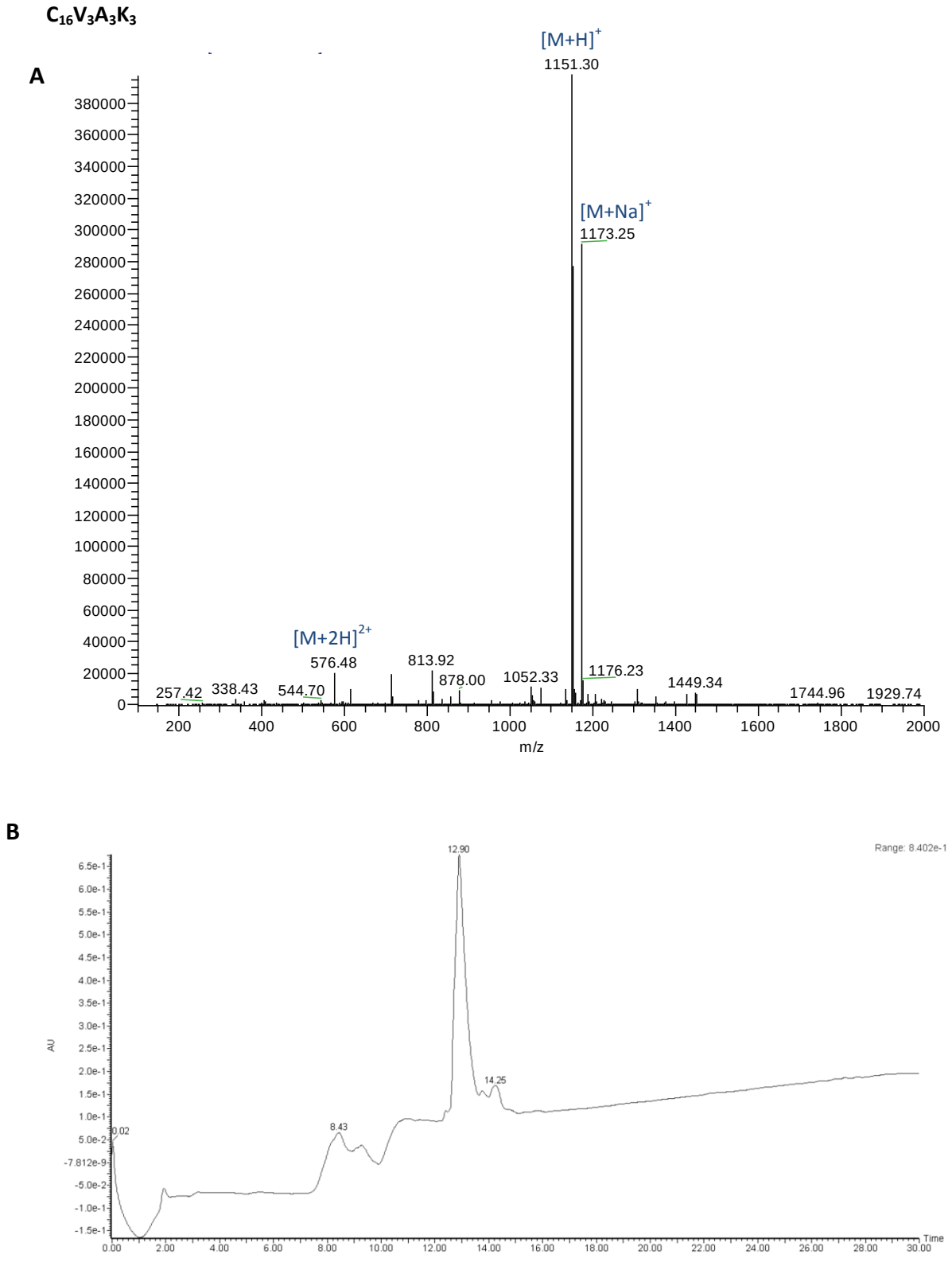


Figure S1. Representative ESI-MS data (A) and analytical HPLC trace, detected at 220 nm (B) of $C_{16}V_3A_3K_3$.

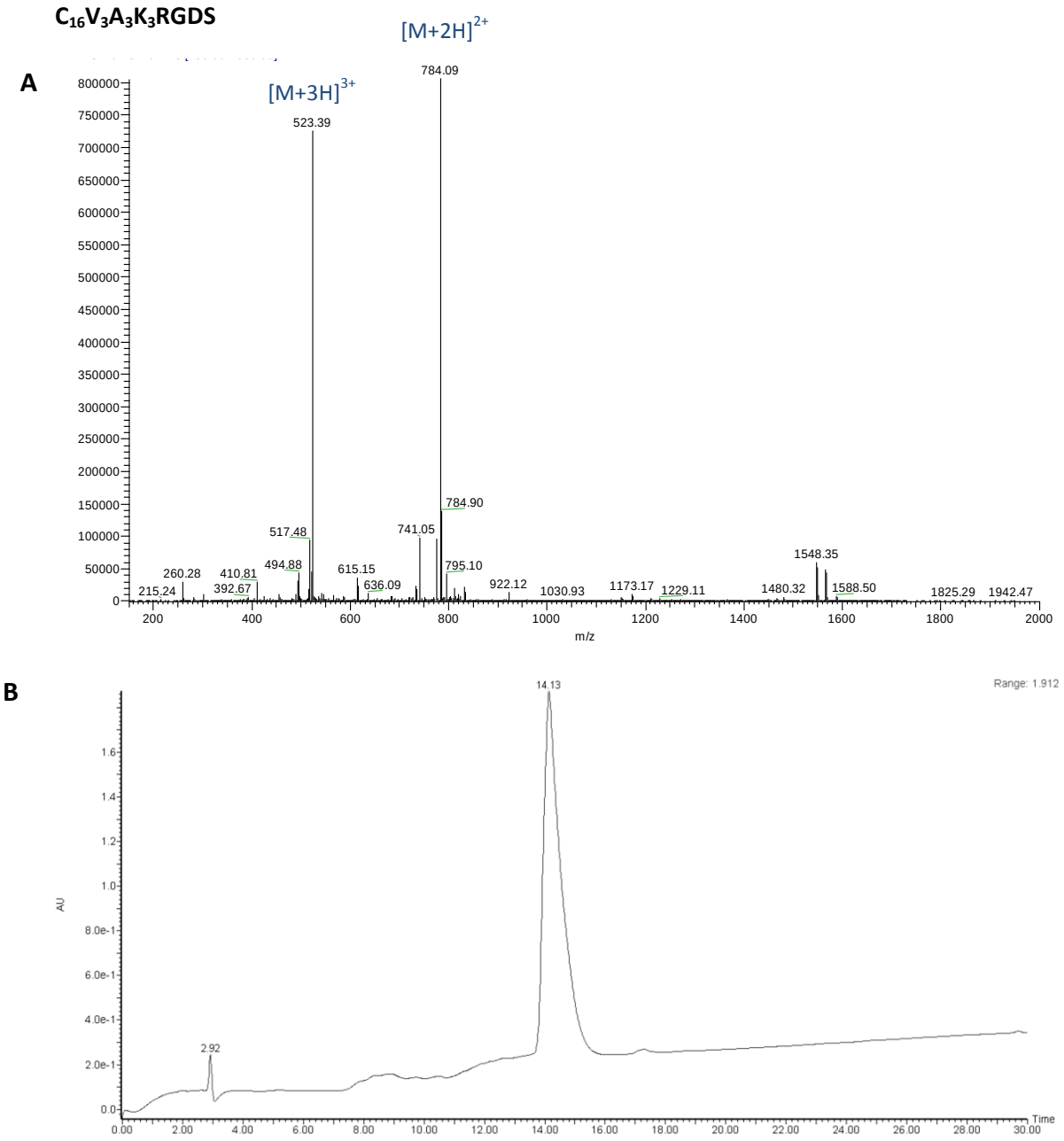


Figure S2. Representative ESI-MS data (A) and analytical HPLC trace, detected at 220 nm (B) of $C_{16}V_3A_3K_3RGDS$.

$C_{16}V_3A_3K_3DGSR$

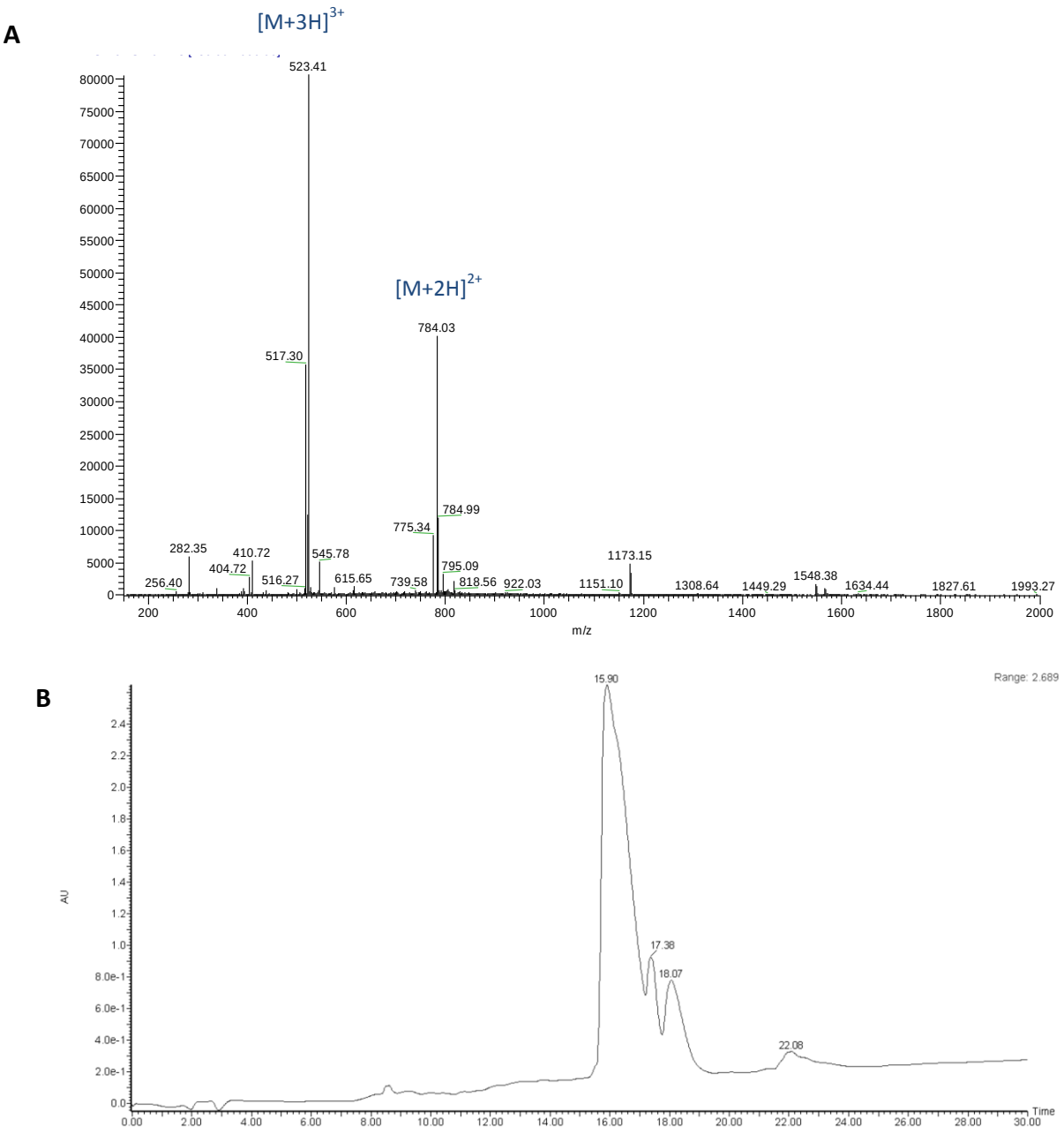
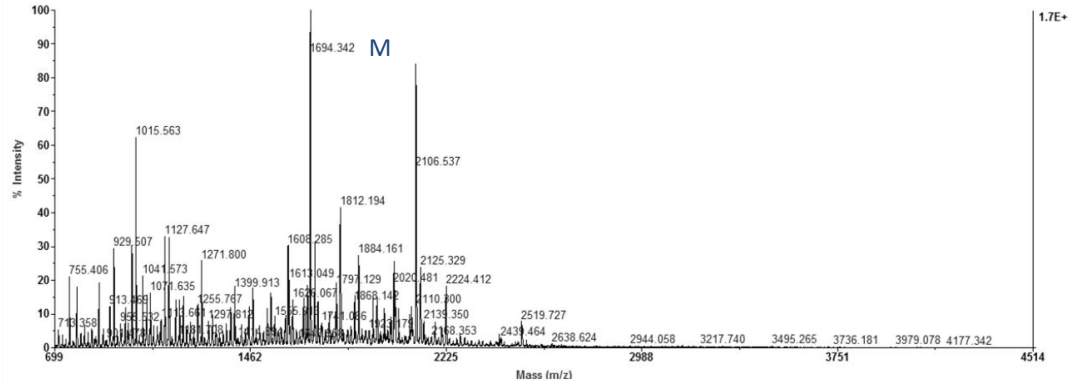


Figure S3. Representative ESI-MS data (A) and analytical HPLC trace, detected at 220 nm (B) of $C_{16}V_3A_3K_3DGSR$.

$C_{16}V_3A_3K_3K_{Rhod}$RGDS-PA

A



B

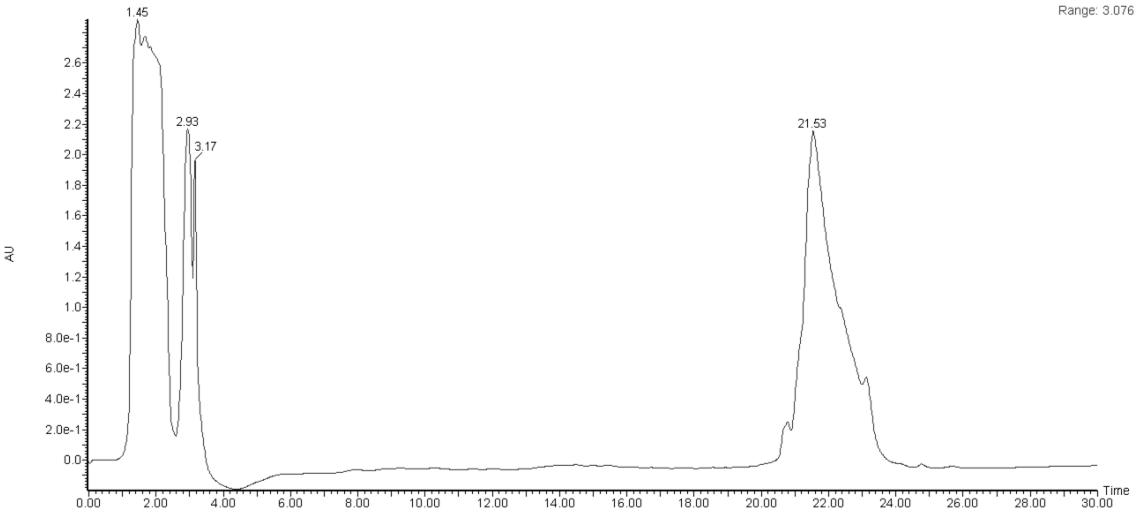


Figure S4. Representative MALDI-MS data (A) and analytical HPLC trace, detected at 220 nm (B) of $C_{16}V_3A_3K_3K_{Rhod}$RGDS-PA.

Microstructure of 50% K_3RGDS-PA membrane

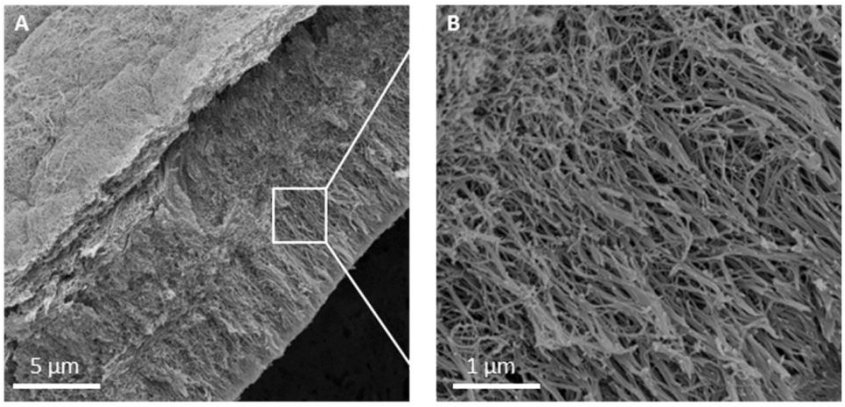


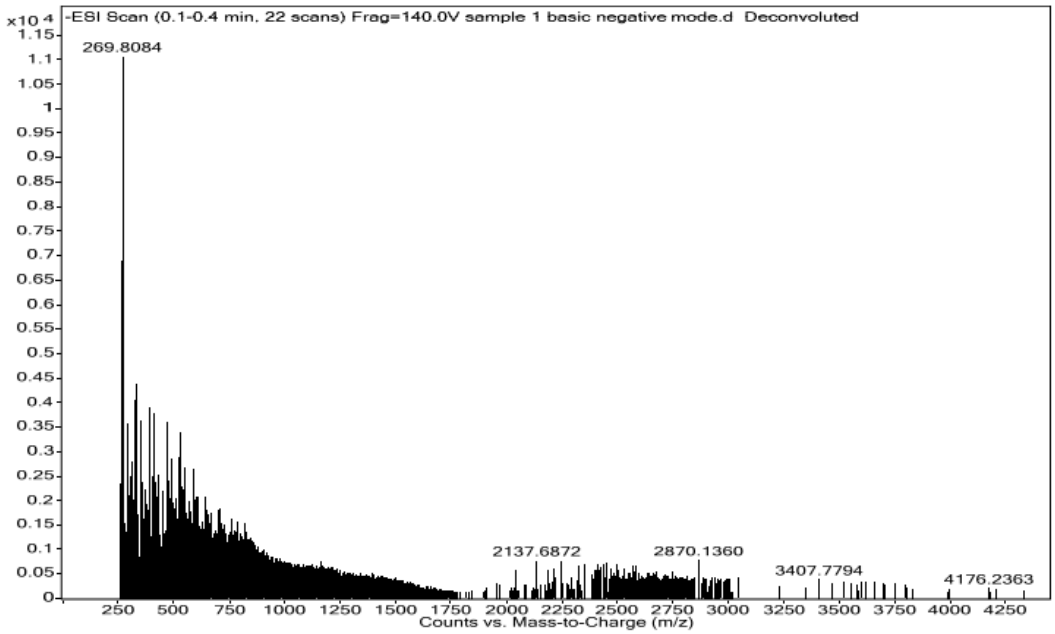
Figure S5. SEM micrographs of the cross section of a 50% K_3RGDS-HA membrane.

Identification of HA oligosaccharides from the enzymatic degradation of PA-HA membranes by mass spectrometry

ESI-MS analysis of 0.1% (w/v) HA solution (control) and of the supernatant after incubating the membranes in 50 U/mL HAase for 14 days (Fig. S6) showed the presence of HA oligosaccharides only for the samples from degradation solutions. Peaks at 331.8, 389.8 and 411.7 were detected corresponding to the masses of HA oligosaccharides with 7, 4 and 13-mers, respectively, with 4 charges m/z= 331.8 $[M_{HA7}\text{-}4H]^{4-}$; 2 charges m/z= 389.8 $[M_{HA4}\text{-}2H]^{2-}$ and with 6 charges m/z= 411.7 $[M_{HA13}\text{-}6H]^{6-}$.

The observed mass for the odd oligosaccharides are fragmentation products of ESI-MS since digestion with HAase produces even-numbered oligosaccharides. However, these fragments were not observed in the control. Therefore, the oligosaccharides found in the degradation solutions may result from the fragmentation of even oligosaccharides originated from the enzymatic digestion.

A



B

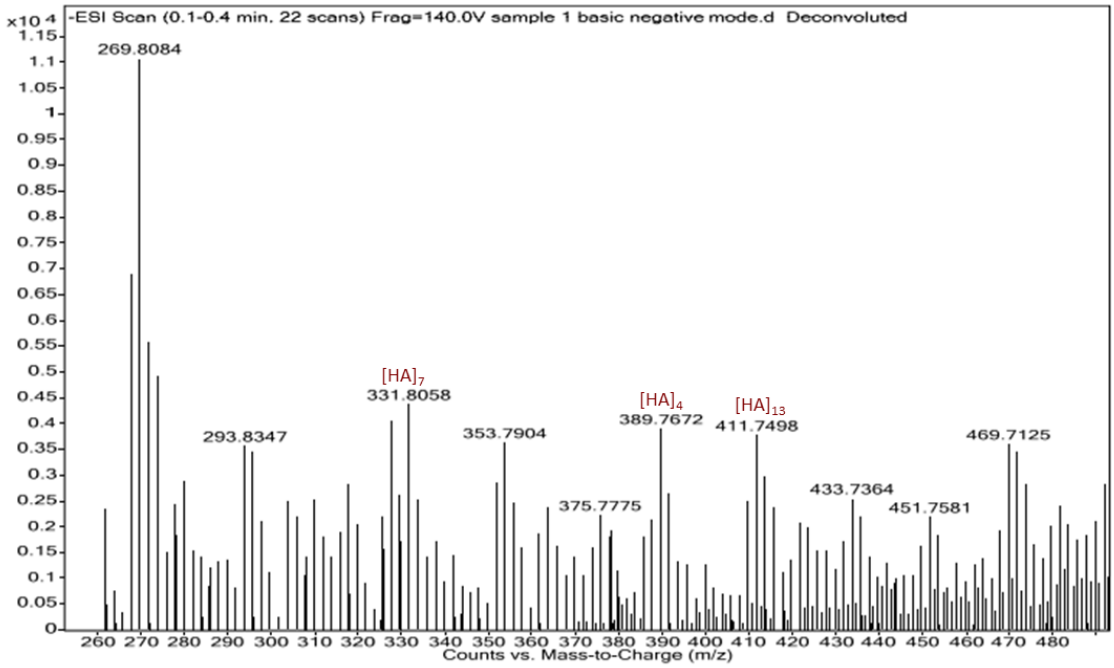


Figure S6. Negative ESI-MS of a 0.1% (w/v) hyaluronan solution (A) and of the supernatant after incubating the membranes in 50 U/mL HAase for 14 days (B).

Morphology of hDFbs cultured on PA-HA membranes in serum-containing medium

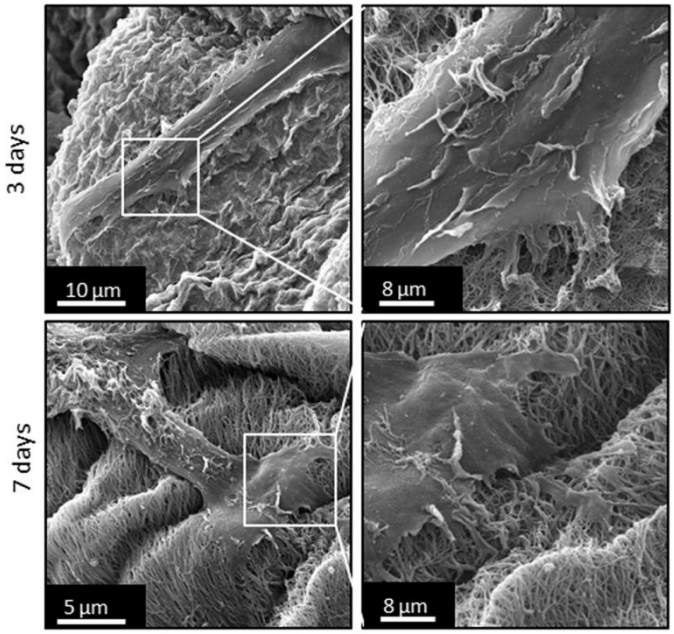


Figure S7. SEM micrographs of hDFbs cultured in K_3-HA membranes. Cells were cultured on the PA side in medium containing 10% FBS up to 7 days.

Analysis of the expression of CD44 receptor on hDFbs by flow cytomety

The expression of CD44 receptor on the surface of hDFbs was assessed by flow cytomety. Briefly, harvested cells were incubated with fluorescent monoclonal antibody against CD44, (BD Biosciences Pharmingen, USA) for 20 minutes at room temperature. Cells were then washed in aquisition buffer (0.1% sodium azide, 1% formaldehyde, in phosphate-buffered saline solution). Unlabeled controls were included to evaluate unspecific binding. Samples were analyzed using a FACScalibur(Becton-Dickinson, USA) with CellQuest analysis software (BectonDickinson, USA).

FACS analysis confirmed a high expression of CD44 (98.67%) on hDFbs. Similar values were reported in the literature for these cells[S3].

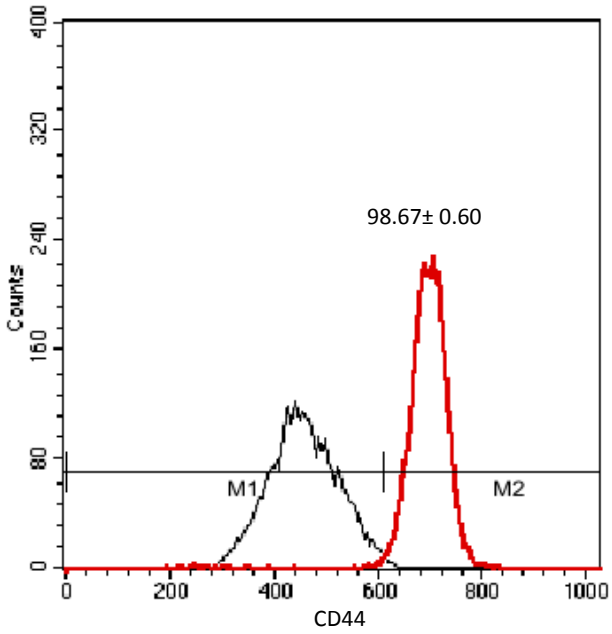


Figure S8 - Flow cytometry analysis of the expression of CD44 on the surface of hDFbs used in the cell culture studies.

hDFbs cultured on hyaluronan-based hydrogel film (non self-assembling material)

To demonstrate the role of the self-assembled component, responsible for the nanofibrillar structure of the self-assembled membranes, on the morphology of adhered cells, hDFbs were cultured on the surface of a hyaluronan hydrogel film (non self-assembling film, control). For that, Corgel® BioHydrogel, a tyramine-substituted HA hydrogel (TS-NaHy 1.5% tyramine substitution, Part #85 Corgel® Kit 1%, Lifecore Biomedical, Inc, Chaska, USA) was prepared according with the supplier's instructions and cells were cultured in serum-free conditions as described in Materials and Methods for the PA-HA membranes. The morphology of the cells was examined by SEM. Corgel® BioHydrogel is a patented hyaluronan hydrogel based on di-hydroxyphenyl linkages of tyramine substituted sodium hyaluronate (NaHy)[S1]and was shown to be biocompatible[S1-S2]. SEM images show that the HA hydrogel films presents a smooth surface (Figure S9-A) with few adherent cells with a small contact area to the surface (Figure S9-B). Furthermore, fibroblasts were completely round, without showing cell protusions (lamellipodia and filopodia) and did not flatten upon contact with the hydrogel surface.

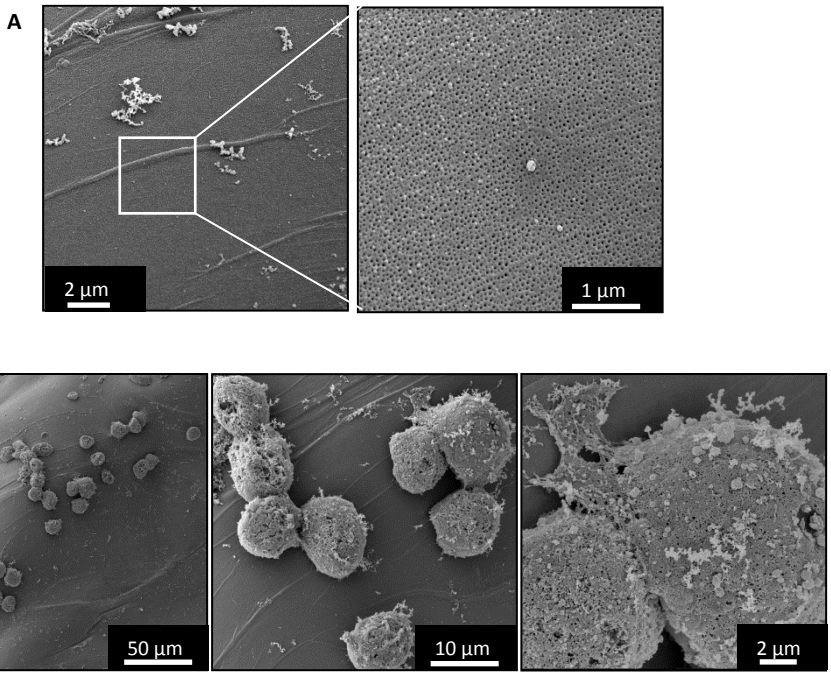


Figure S9. SEM micrographs of TS-NaHy hydrogel surface (A) and morphology of hDFbs cultured on their surface in serum-free medium for 24 h.

References

S1. A. Darr, A. Calabro, Synthesis and characterization of tyramine-based hyaluronan hydrogels. J Mater Sci: Mater Med 2009; 20: 33-44

S2. L. Chin, A. Calabro, E. E. Rodriguez, C. D. Tan, E. Walker, K. A. Derwin. Characterization of and host response to tyramine substituted-hyaluronan enriched fascia extracellular matrix. J Mater Sci: Mater Med 2011; 22:1465-1477

S3. A. Blasi, C. Martino, L. Balducci, M. Saldarelli, A. Soleti, S. Navone, L. Canzi, S. Cristini, G. Invernici, E. Parati, G. Alessandri. Dermal fibroblasts display similar phenotypic and differentiation capacity to fat-derived mesenchymal stem cells, but differ in anti-inflammatory and angiogenic potential. Vascular Cell 2011, 3:5