Supplementary Data

Supplementary Materials and Methods

Specific enzymatic digestion of peptide amphiphiles

The matrix metalloprotease 1 (MMP1, ab134442; Abcam) and 7 (MMP7, 10277-H01H; Sino Biological, Life Technologies) proenzymes were incubated in 50 mM Tris-HCl, pH 7.5 containing 0.5 mM *p*-aminophenylmercuric acetate (APMA; Sigma-Aldrich) for 3 h at 37°C for activation. Self-assembled $_{mf}$PA and $_{mf}$PA:$_{d}$PA were prepared at 1.25×10^{-3} M in 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 5 mM $CaCl_2$, 0.01% Brij-35, and 50 μM APMA and incubated with activated enzymes for 12 h at 37°C. Enzyme activity was confirmed by casein gel zymography (Supplementary Fig. S8), as described below.

Mass spectrometry analysis

Peptide amphiphile (PA) samples resulting from specific enzyme cleavage assay were diluted 1:100 with high-performance liquid chromatography degree water for mass spectrometry analysis. The peaks were identified using a Bruker MicroTof QII high-resolution time-of-flight mass spectrometer equipped with an electrospray ionization source. The ion spray voltage was held at 4500 V in a positive ion mode. The nebulizer gas was nitrogen with a pressure of 1.0 bar. Drying gas and temperature were 8 L/min and data were acquired over a mass range of 50–3000 Da. The amino acid sequences were obtained using Data Analysis version 4.0 SP1 Bruker sequence editor software and Mass Analysis Peptide Sequence Prediction.

Critical aggregation concentration analysis

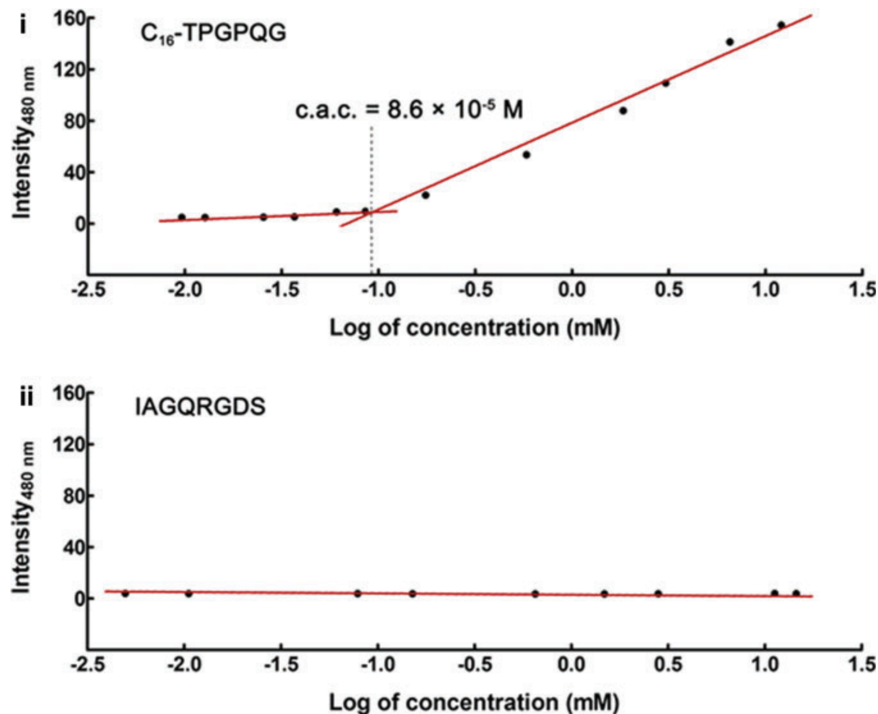
Fluorescence spectra were recorded with a Varian Cary Eclipse Fluorescence Spectrometer with samples in 4-mm inner width Quartz cuvettes. For the ThT assay, the spectra were recorded from 460 to 600 nm using an excitation wavelength $\lambda_{ex} = 440$ nm, and the peptides were dissolved in a 5.0×10^{-3} wt% ThT solution.

Fourier transform infrared spectroscopy

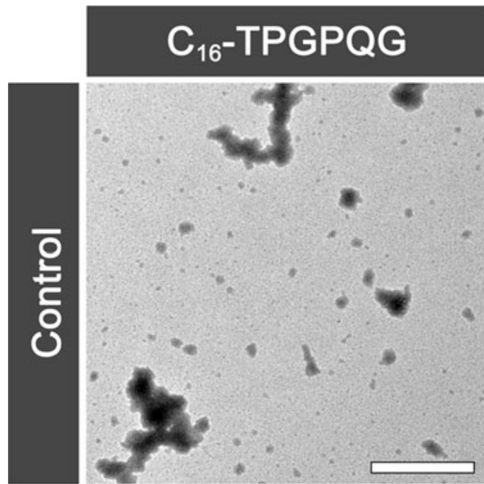
The secondary structure of $_{mf}$PA aggregates was studied by Fourier transform infrared (FTIR) spectroscopy in the amide I region. Spectra were recorded using a Nexus FTIR spectrometer equipped with a DTGS detector and a multiple reflection attenuated total reflectance system. The analyzed solutions were sandwiched in ring spacers between two CaF_2 plate windows (spacer 0.025 mm thick). All spectra were scanned 128 times over the range of 4000–950 cm^{-1}. Data were corrected by baseline subtraction.

Transmission electron microscopy

Preparation of PAs in solution and tissue constructs for transmission electron microscopy (TEM) imaging was performed with reagents obtained from Elektron Technologies.

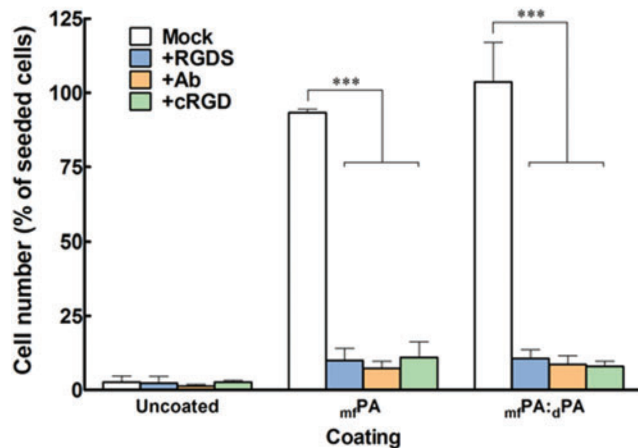


SUPPLEMENTARY FIG. S1. ThT fluorescence spectroscopy of predicted cleavage products from $_{mf}$PA. Critical aggregation concentration (c.a.c.) was determined for synthesized C_{16}-TPGPQG **(i)** at the intersection of the regression curves (*red lines*), whereas IAGQRGDS did not self-aggregate **(ii)**.



SUPPLEMENTARY FIG. S2. Transmission electron micrograph of synthetic C_{16}-TPGPQG in aqueous solution. At 1.25×10^{-3} M, this molecule self-assembled and formed micelle-like structures similar to those observed after enzymatic digestion of $_{mf}$PA-containing nanotapes with matrix metalloprotease 1 (MMP1) (Fig. 2a). Scale bar, 500 nm.

Droplets of PA solutions were placed on carbon-coated Cu grids, stained with 2 wt% uranyl acetate acid, and dried. For tissues, fixation was performed in freshly prepared 2:2.5% paraformaldehyde:glutaraldehyde in 25 mM HEPES buffer pH 7.4 overnight at 5°C. Buffer-washed tissue was then stained in 1% osmium tetroxide, washed again (3×15 min), dehydrated through 30–100% acetone series, and embedded in freshly prepared resin comprising 0.3:2:4:5 v/v benzyl dimethylamine:methyl nadic anhydride:dodecenyl succinic anhydride:Agar 100 at 60°C. Tissue cross sections, 70 nm thick, were impregnated with 2 wt% uranyl acetate acid for 1 h before imaging using a Philips CM20 TEM microscope operated at 200 kV. Evaluation of TEM micrographs and calculation of the average nanotape width and collagen diameter and *d*-spacing were performed after 100 measurements using the ImageJ v1.46 software.



SUPPLEMENTARY FIG. S3. Specific adhesion of human corneal stromal fibroblasts (hCSFs) cultured on $_{mf}$PA and $_{mf}$PA:$_{d}$PA films coating low-attachment plates. Cells were incubated with soluble $_{d}$PA (mock, *white*), $_{sf}$PA (+RGDS, *blue*), anti-αVβ5 integrin antibody (+Ab, *orange*), and cyclic RGD (cRGD, *green bars*) before seeding to block integrin-mediated adhesion to the surfaces coated with $_{mf}$PA or $_{mf}$PA:$_{d}$PA. Uncoated surfaces were used as negative adhesion controls. All experiments were performed using low-attachment surfaces to maximize specificity of adhesion. Mean ± standard deviation, $n = 3$ for all experiments; *** corresponded to $p < 0.001$.

Cell culture

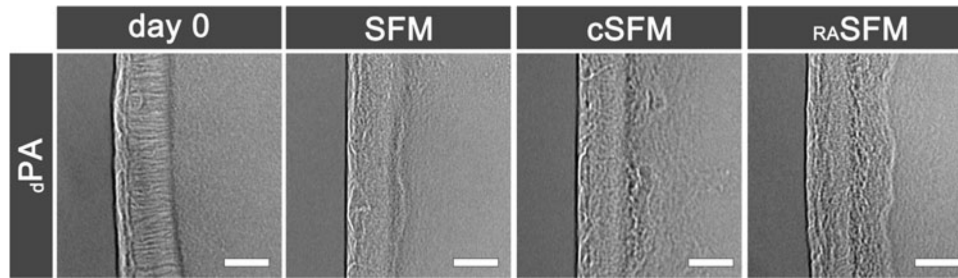
To isolate human corneal stromal fibroblasts, corneal tissue was shredded using a scalpel, transferred to 1:1 Dulbecco's modified Eagle's medium: Ham's F12 (DMEM/F12) supplemented with 2 g/L of collagenase type I (Life Technologies) and 5% fetal bovine serum (FBS; Biosera), and incubated under rotation for 5 h at 37°C, followed by incubation with 0.25% Trypsin-EDTA in DMEM/F12 for 10 min. Isolated human corneal stromal fibroblasts (hCSFs) were plated onto standard polystyrene culture plates (Nunc; Thermo Scientific) and maintained in DMEM/F12 media supplemented with 5% FBS, 1×10^{-3} M ascorbic acid, and 1% penicillin/streptomycin at 37°C and 5% CO_2. Media were replaced every 2–3 days. Upon reaching 70–80% confluence, cells were passaged and transferred to serum-free media (SFM) 3 days before subsequent experiments to inactivate hCSFs. Adult, human dermal fibroblasts purchased from Life Technologies were mainlined at 37°C until confluent. Cell cultures were monitored using a Nikon Eclipse inverted microscope (Nikon) coupled with a Jenoptik CCD camera (Jenoptik AG).

Adhesion and proliferation assays

Cell proliferation was quantified using the AlamarBlue assay. Briefly, cell media were replaced 24 h postseeding with resazurin reagent (diluted 1:10 in SFM). After incubating for 4 h at 37°C, resazurin was removed and sampled (0.1 mL, in triplicate) for fluorescence emission at 590 nm and cells replenished with fresh culture media. The process was repeated for each time point evaluated. Cell number was calculated by interpolation using a standard curve for the fluorescence values of 1, 5, 10, 20, 50, and 100×10^3 cells.

Analysis of gene marker expression

RNA quality was assessed using a NanoDrop 2000 spectrophotometer (Thermo Scientific) to ensure the 260/280 ratio was within the range 1.7–2.0. Synthesis of cDNA from isolated total RNA was performed using the Maxima First cDNA Synthesis kit (Thermo Scientific) according to the manufacturer's instructions in a TcPlus thermocycler (Techne). Quantitative real-time PCR was performed using the default thermal profile of the Eco Real-Time PCR System (Illumina), with the following 40 × three-step cycle: 10 s denaturation, 95°C; 30 s annealing, 60°C; 15 s elongation, 72°C. The relative expression of genes coding for keratocan (*KERA*), lumican (*LUM*), decorin (*DCN*), aldehyde dehydrogenase 1 A1 (*ALDH1*), *MMP1*, and α-smooth muscle actin (*ACTA2*) was analyzed in duplicate, calculated by the comparative threshold cycle (Eco Software v3.1;



SUPPLEMENTARY FIG. S4. Bright-field micrographs of $_{d}$PA coatings. Surfaces dry-spotted with $_{d}$PA produced a continuous coating (day 0) that remained intact after the 7-day incubation with MMP-free (serum-free media [SFM] and $_{RA}$SFM) or MMP-containing culture media (cSFM). Scale bars, 20 μm.

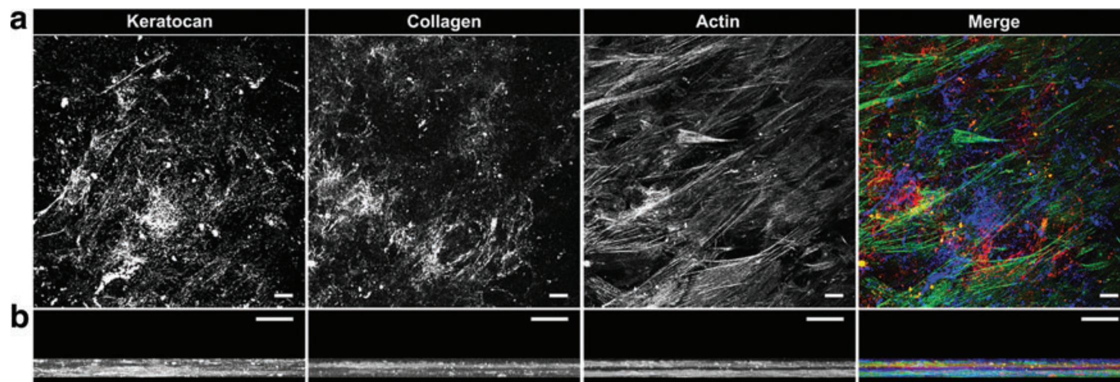
Illumina), and normalized to the expression of the RNA polymerase II subunit RPB1 (*POLR2A*) housekeeping gene in three independent experiments. The primers used to evaluate the expression of *KERA* (fwd. TATTCCTGG AAGGCAAGGTG, rev. ACCTGCCTCACACTTCTAGACC), *LUM* (fwd. CCTGGTTGAGCTGGATCTGT, rev. TAGG ATAATGGCCCCAGGA), *DCN* (fwd. AATTGAAAATG GGGCTTTCC, rev. CTGCTGATTTTGTTGCCATC), *ALDH1* (fwd. CTCTCACTGCTCTCCACGTG, rev. GAGAAGAA ATGGCTGCCCCT), *MMP1* (fwd. AGGTCTCTGAGGG TCAAGCA, rev. CTGGTTGAAAAGCATGAGCA), *ACTA2* (fwd. CTGAGCGTGGCTATTCCTTC, rev. TTCTCAAGG GAGGATGAGGA), and *POLR2A* (fwd. CATCATCCGA GACAATGGTG, rev. AACAATGTCCCCATCACACA) were validated for specificity and efficiency.

Analysis of MMP activity

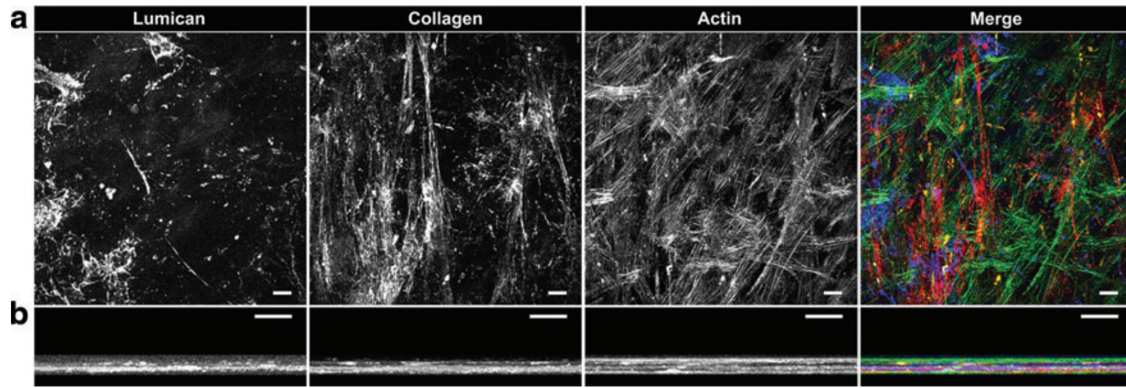
The presence of MMPs in hCSF culture supernatants was evaluated using 12% Ready Gel casein zymogram precast gels (Bio-Rad). Briefly, 5 mL samples obtained from culture supernatants in different conditions and at various time points (before seeding: cSFM; with retinoic acid [RA] supplement: day 3 to 90; without RA supplement: +1, +2, and +3; with RA supplement after reattachment: R1) were concentrated 200× by repeated ultrafiltration using Vivaspin 500 columns (3 kDa cutoff; GE Healthcare), mixed with loading buffer, and run by nonreducing sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS/PAGE). Gels were then washed in 2.5% Triton-X 100 for 3 × 15 min, incubated with agitation in 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 5 mM $CaCl_2$, and 0.01% Brij-35 for 12 h at 37°C, stained with Coomassie G-250 (Sigma-Aldrich) for 1 h, and finally washed with methanol/acetic acid solution overnight before imaging.

Microscopy analysis

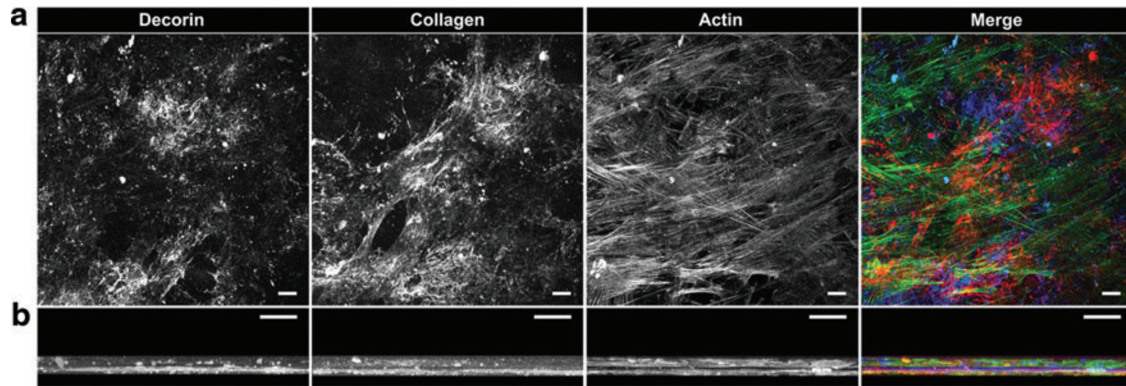
Tissues were fixed in 4% paraformaldehyde for 20 min, washed twice with phosphate-buffered saline (PBS) for 5 min, blocked for 1 h in PBS supplemented with 2% bovine serum albumin, and incubated with rabbit anticollagen type I antibody (ab292; Abcam) and antikeratocan, antilumican (sc-66941 and sc-166871, respectively; Santa Cruz Biotechnology), or antidecorin (PC673, CalBiochem; Millipore) antibodies diluted 1:500 in blocking solution for 2 h, washed thrice with PBS for 5 min, and incubated with corresponding Alexa Fluor 405 and 594-conjugated secondary antibodies and Alexa 488-conjugated phalloidin (A12379; Life Technologies) for 1 h and imaged using an SP2 confocal microscope (Leica). Micrographs were analyzed using ImageJ v1.46.



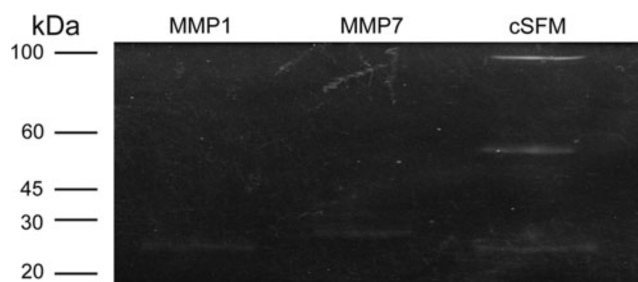
SUPPLEMENTARY FIG. S5. Expression of keratocan and collagen type I in self-detached, bioprosthetic corneal tissue constructs. **(a)** Representative *top* view and **(b)** *z*-stack view of corneal tissues fabricated by hCSFs grown on $_{mf}$PA:$_{d}$PA templates for 90 days. Tissues incubated with antikeratocan (*blue*) and anticollagen type I antibodies (*red*) and phalloidin (*green*) were imaged by confocal fluorescence microscopy, and specific signals were analyzed in individual color channels. Merged image corresponds to the *left panel* from Figure 8. Scale bars, 20 μm.



SUPPLEMENTARY FIG. S6. Expression of lumican and collagen type I in self-detached, bioprosthetic corneal tissue constructs. **(a)** Representative *top* view and **(b)** *z*-stack view of corneal tissues fabricated by hCSFs grown on $_{mf}$PA:$_{d}$PA templates for 90 days. Tissues incubated with antilumican (*blue*) and anticollagen type I antibodies (*red*) and phalloidin (*green*) were imaged by confocal fluorescence microscopy, and specific signals were analyzed in individual color channels. Merged image corresponds to the *central panel* from Figure 8. Scale bars, 20 μm.



SUPPLEMENTARY FIG. S7. Expression of decorin and collagen type I in self-detached, bioprosthetic corneal tissue constructs. **(a)** Representative *top* view and **(b)** *z*-stack view of corneal tissues fabricated by hCSFs grown on $_{mf}$PA:$_{d}$PA templates for 90 days. Tissues incubated with antidecorin (*blue*) and anticollagen type I antibodies (*red*) and phalloidin (*green*) were imaged by confocal fluorescence microscopy, and specific signals were analyzed in individual color channels. Merged image corresponds to the *right panel* from Figure 8. Scale bars, 20 μm.



SUPPLEMENTARY FIG. S8. Activity of recombinant MMPs. Recombinant MMP1 and MMP7 were analyzed by zymography using 12% casein gels in parallel with hCSF-conditioned medium (cSFM). Gel clearance was observed in the 25 and 27 kDa regions expected for MMP1 and MMP7, respectively, indicating that both enzymes were active.