ELECTRONIC SUPPLEMENTARY INFORMATION

Engineering a Macroporous Fibrin-based Sequential Interpenetrating Polymer Network for Dermal Tissue Engineering

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^{1}H NMR spectrum of 4k PEGDM

Fig. S1 depicts a typical ^{1}H nuclear magnetic resonance (NMR) spectrum of 4k PEGDM. The expected peaks[1] were observed for PEGDM and the degree of methacrylation was determined to be approximately 100%.

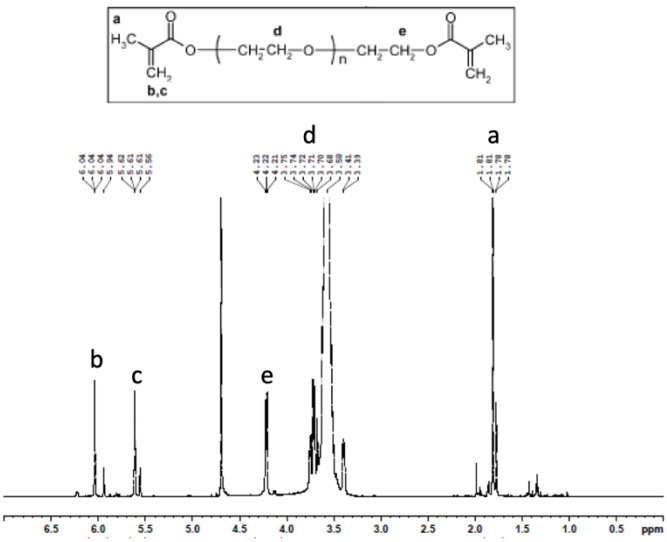

Fig. S1 1H NMR spectrum of 4k PEGDM.

Quantification of free amino groups in SA/SAM

A 2,4,6-trinitrobenzene sulfonic acid (TNBS) assay was performed to evaluate the degree of functionalization of SAM. Serial dilutions (from 0.1 to 2 mg/ml) of SA and SAM solutions were prepared in borate buffer (250 mM, pH 9). A total of 10 µL of TNBS solution at 5% (w/v) was added to 250 µl of each sample and incubated for 5 min at room temperature (RT). Next, 1 ml of stop buffer (200 mM NaH_2PO_4, 3 mM $NaSO_3$) was added to each solution. Finally, the absorbance was then measured at 420 nm using a spectrometer (Fig. S2). Approximately 67% of amino groups on SA were conjugated with methacrylate residues.

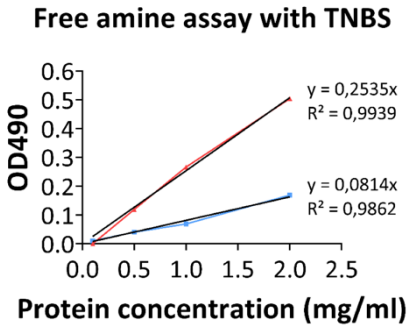

Fig. S2. Free amine assay with the TNBS assay. The degree of functionalization of SAM was determined using a TNBS assay. The free amine proportion was calculated as the ratio between the slope of the SAM trendline (red) to the slope of the SA trendline (blue).

Determination of the optimal strain

The small-amplitude oscillatory shear (SAOS) technique was used to characterize the rheological properties across the different gel formulations as reported by others.[2] This technique consists of two parallel discs, one of which rotates. The gel sample was confined between the two shearing plates and subjected to a small-amplitude torsional oscillation. Strain sweeps from 0.1 to 10% strain were conducted at a frequency of 1 Hz (chosen arbitrarily) in order to determine a suitable strain for subsequent time sweeps. The strain must be small enough to be in the linear-viscoelastic region. The tests were performed either at RT (PEGDM-*co*-SAM hydrogels) or at 37°C (fibrin networks) on fully formed and equilibrated gels in PBS.

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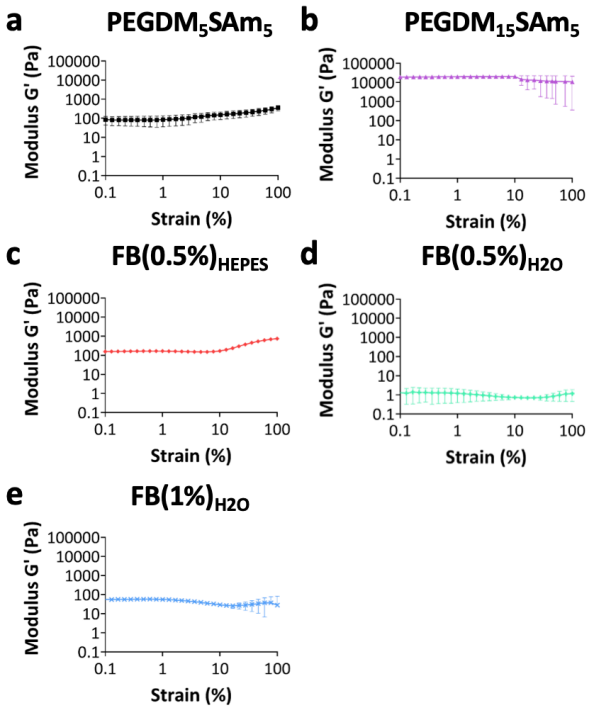

Fig. S3. Strain sweeps. The storage modulus (G') was determined from 0.1 to 100% strain for (a) $PEGDM_5SAM_5$, (b) $PEGDM_{15}SAM_5$, (c) $Fb(0.5\%)_{HEPES}$, (d) $Fb(0.5\%)_{H20}$ and (e). $Fb(1\%)_{H20}$. Values represent mean (n≥ 3) ± standard deviation (SD).

The test geometry was a 50 mm diameter plate. Each sample was sandwiched between the two plates and kept hydrated. The top plate was lowered down to the required distance between the parallel plates (gap), which was 1 mm for all the tests. $PEGDM_5SAM_5$ formulation had a linear strain region up to 1.3% strain (Fig. S3a). $PEGDM_{15}SAM_5$ formulation showed linear behavior for the storage modulus (G') up to 10% strain (Fig. S3b). $Fb(0.5\ \%)_{HEPES}$ exhibited a linear region up to 10% strain (Fig. S3c). $Fb(0.5\%)H_20$ formulation had a linear region up to 13% strain (Fig. S3d). $Fb(1\%)H_20$ had a linear region up to 1.7% strain (Fig. S3e). Therefore, we chose a strain amplitude of 1% for subsequent sweeps as it was within the linear region across all formulations tested.

Determination of the optimal frequency

Frequency sweeps from 0.5 to 10 Hz were subsequently conducted at a constant strain of 1% (determined previously with the strain sweep protocol). Each sample was sandwiched between the two plates and kept hydrated. The top plate was lowered down to the required distance between the parallel plates (gap), which was 1 mm for all the tests. The tests were performed on fully formed and equilibrated gels in PBS. The two PEGDM-*co*-SAM and the $Fb(0.5\%)_{HEPES}$ formulations exhibited a plateau for all the frequencies measured (Fig. S4a, b and c, respectively). The gel region was observed until an approximate frequency of 10 Hz for $Fb(0.5\%)_{H20}$ (Fig. S4d) and until an approximate frequency of 7 Hz for $Fb(1\%)_{H20}$ (Fig. S4e). A frequency of 1 Hz was therefore chosen for subsequent time sweep assays.

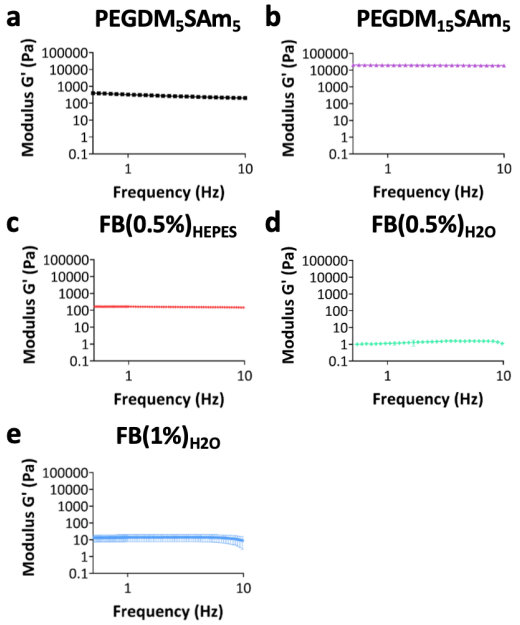

Fig. S4. Frequency sweeps. G' was determined from 0.5 to 10 Hz for (a) $PEGDM_5SAM_5$, (b) $PEGDM_{15}SAM_5$, (c) $Fb(0.5\%)_{HEPES}$, (d) $Fb(0.5\%)_{H20}$ and (e) $Fb(1\%)_{H20}$. Values represent mean ± SD (n≥ 3).

Determination of the gelation point

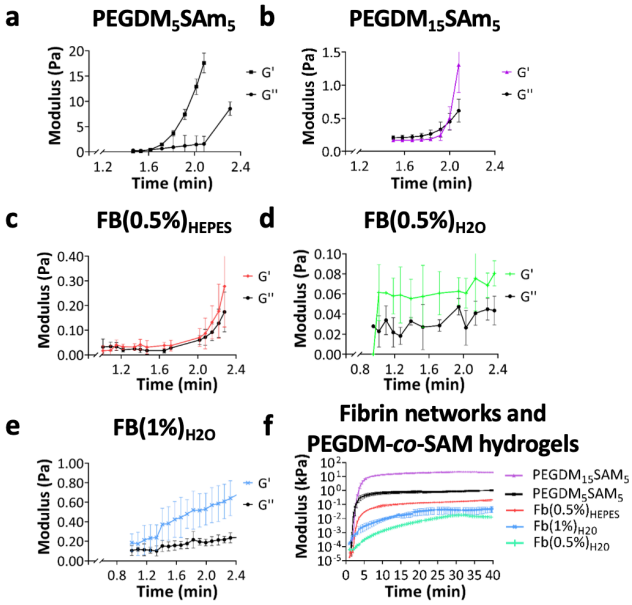

Fig. S5. The gelation point was determined for each formulation: (a) $PEGDM_5SAM_5$, (b) $PEGDM_{15}SAM_5$, (c) $Fb(0.5\%)_{HEPES}$, (d) $Fb(0.5\%)_{H20}$ and (e) $Fb(1\%)_{H20}$. Storage moduli (G') were also measured as a function of time for PEGDM-*co*-SAM based fully-formed hydrogels and fibrin networks (f). Values represent mean ± SD (n≥ 3).

The gelation point occurs at the time at which the storage modulus (G') and loss modulus (G") cross each other. $PEGDM_5SAM_5$ reached the gelation point before $PEGDM_{15}SAM_5$ (1.6 vs 2 min) (Fig. S5a, b) whereas G' (i.e., elastic or storage modulus) plateaued within 10 min for both formulations (Fig. S5e). For fibrin hydrogels, $Fb(1\%)_{H20}$ polymerized faster than the other fibrin formulations,

with a gel point at < 1 min vs approximately 1.2 and 1 min for $Fb(0.5\%)_{HEPES}$ and $Fb(0.5\%)_{H2O}$, respectively (Fig. S5e, c and d). The three fibrin formulations formed stable hydrogels and their G' plateaued within 10 min for $Fb(0.5\%)_{HEPES}$, 20 min for $Fb(1\%)_{H2O}$ and 38 min for $Fb(0.5\%)_{H2O}$ (Fig. S5e).

Cell morphology of HDFs in Fibrin/PEGDM-*co*-SAM sequential IPN hydrogels

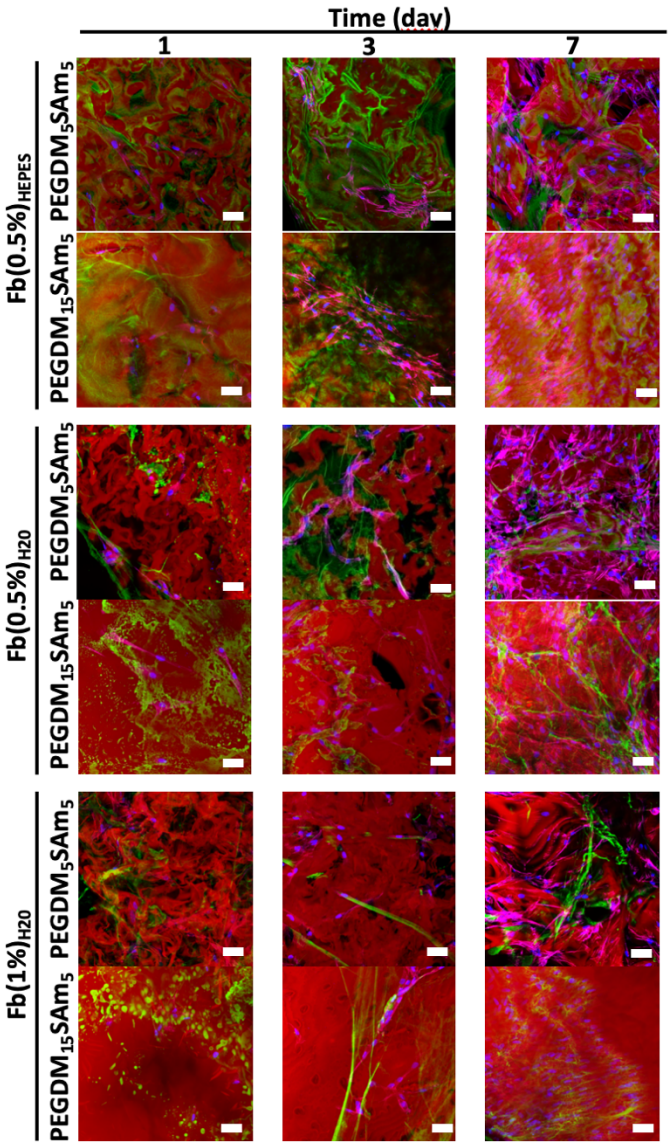

Fig. S6. Fibrin/PEGDM-*co*-SAM sequential IPN hydrogels. HDFs were cultured in various Fibrin/PEGDM-*co*-SAM IPN constructs: Fibrin/$PEGDM_5SAM_5$ and Fibrin/$PEGDM_{15}SAM_5$ fabricated with different fibrin formulations: $Fb(0.5\%)_{HEPES}$, $Fb(0.5\%)_{H2O}$ or $Fb(1\%)_{H2O}$. Cell morphology was analyzed by confocal microscopy after 1, 3, and 7 d of culture depicting F-actin (dark pink), nuclei (blue), fibrin (green) and PEGDM-*co*-SAM (red). Scale bars = 50 µm.

Human dermal fibroblasts (HDFs) seeded at $2x10^4$ cells/gel were cultured within the Fibrin/PEGDM-*co*-SAM IPN hydrogels: $PEGDM_5SAm_5$ and $PEGDM_{15}SAm_5$-based IPNs made with $Fb(0.5\%)_{HEPES}$, $Fb(0.5\%)_{H20}$ or $Fb(1\%)_{H20}$. Cell morphology was subsequently imaged at day 1, 3 and 7 (Fig. S6).

References

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