Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Clickable decellularized extracellular matrix as a new tool for building hybrid-hydrogels to model chronic fibrotic diseases in vitro

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Supporting Information

Small molecule and macromer synthesis.

Synthesis of ethyl2-(bromomethyl) acrylate. The product was verified by proton nuclear magnetic resonance (NMR) performed on a Bruker Avance-III 300 NMR Spectrometer (7.05 T) at the University of Colorado, Boulder for every reaction. The average functionalization was approximately 96% calculated by summing peak integration values and dividing by the 9 expected hydrogens. Product with functionalization greater than 90% was used in the synthesis of PEGαMA. A representative NMR spectrum can be seen in Fig. S1.

Synthesis of poly(ethylene glycol)-alpha methacrylate. Product functionalization was verified by proton NMR. The degree of vinyl end group functionality was calculated using a ratio of the integration area of the proton resonance peak of $C=CH_2$ to that of the poly(ethylene glycol) (PEG) backbone. The representative NMR spectrum in Fig. S2 shows >98% functionality based on the integration ratio of peak C to the peak representing the PEG backbone.

Synthesis of poly(ethylene glycol)-methacrylate. This protocol was adapted from a previously reported version.[1, 2] PEG-hydroxyl (8-arm, 10 kg/mol; JenKem Technology) was dissolved in anhydrous tetrahydrofuran (THF; Sigma Aldrich) and purged with argon. Triethylamine (4 equivalents with respect to hydroxyls, TEA; Thomas Scientific) was injected through a septum into the reaction vessel at 4x molar excess to PEG-hydroxyl groups. Then, methacryloyl chloride (Acros Organics) was added dropwise at

4x molar excess with respect to hydroxyls, and the reaction was stirred for 48 h at room temperature before being filtered through Celite 545 to remove quaternary ammonium salts. The solution was then concentrated by rotary evaporation and precipitated and washed 3x in ice-cold diethyl ether. The product was dried under vacuum overnight. The product was verified by proton NMR (Fig. S3). $_1$H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.8 (t, 3H, CH_3–), 3.62 (s, 114H, PEG backbone), 4.17-4.21 (m, 2H, –CH_2–C(O)–O–O) 5.60 (t, 1H, –C=CH_2), 6.0 (d, 1H, –C=CH_2).

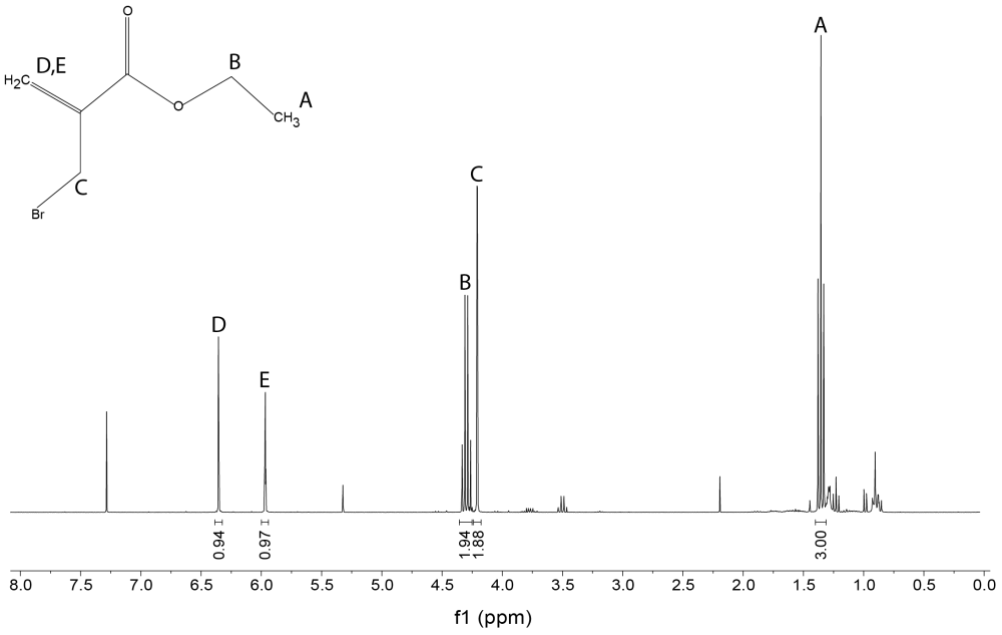

Fig. S1 H_1 NMR spectrum of EBrMa ($CDCl_3$, 300 MHz). Percent functionalization was 97.6%. $_1$H-NMR (300 MHz, $CDCl_3$): δ (ppm) 1.3 (t, 3H, –CH_3), 4.16 (s, 2H, –CH_2–Br), 4.25 (q, 2H, –CH_2–O–), 5.9 and 6.3 (s, 1H, =CH_2).

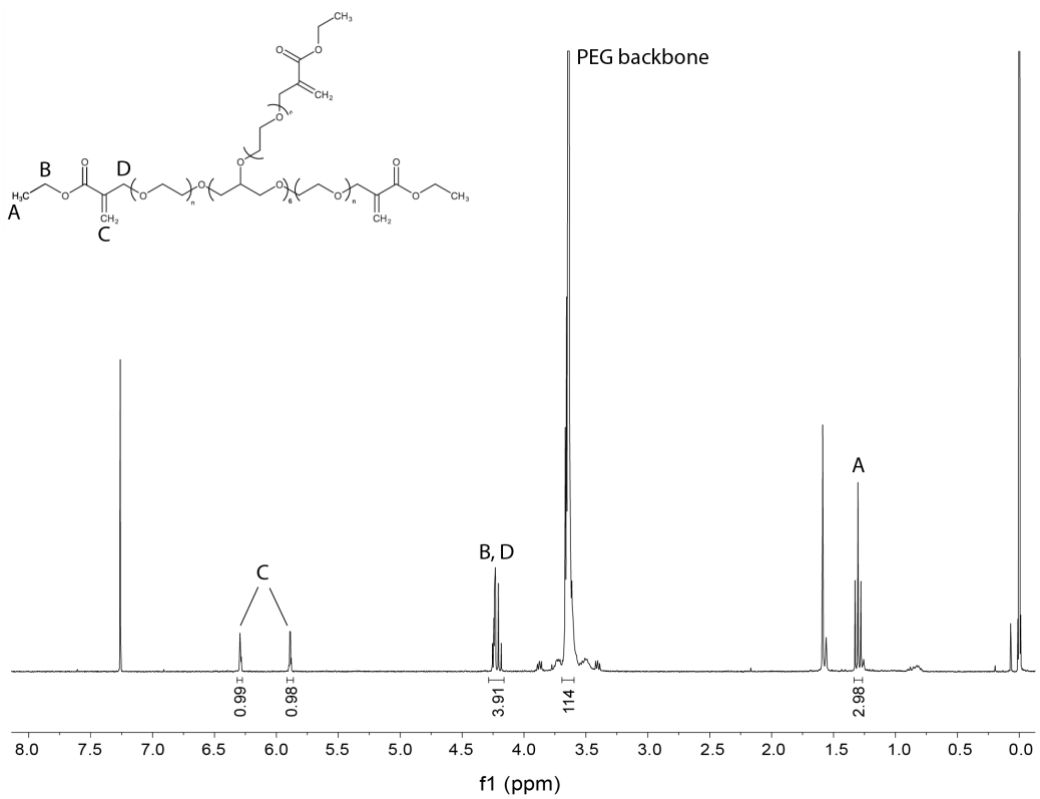

Fig. S2 H_1 NMR spectrum of PEGαMA ($CDCl_3$, 300 MHz). The degree of vinyl end group, $C=CH_2$, calculated by the integration ratio of peak C (2H), compared to the peak of the PEG backbone. $_1H$ NMR (300 MHz, $CDCl_3$): δ (ppm) 1.23 (t, 6H, CH_3–), 3.62 (s, 114H, PEG backbone), 4.17-4.21 (t, s, 8H, –CH_2–C(O)–O–O, –O–CH_2–C(=CH_2)–), 5.90 (s, 1H, –C=CH_2), 6.31 (s, 1H, –C=CH_2).

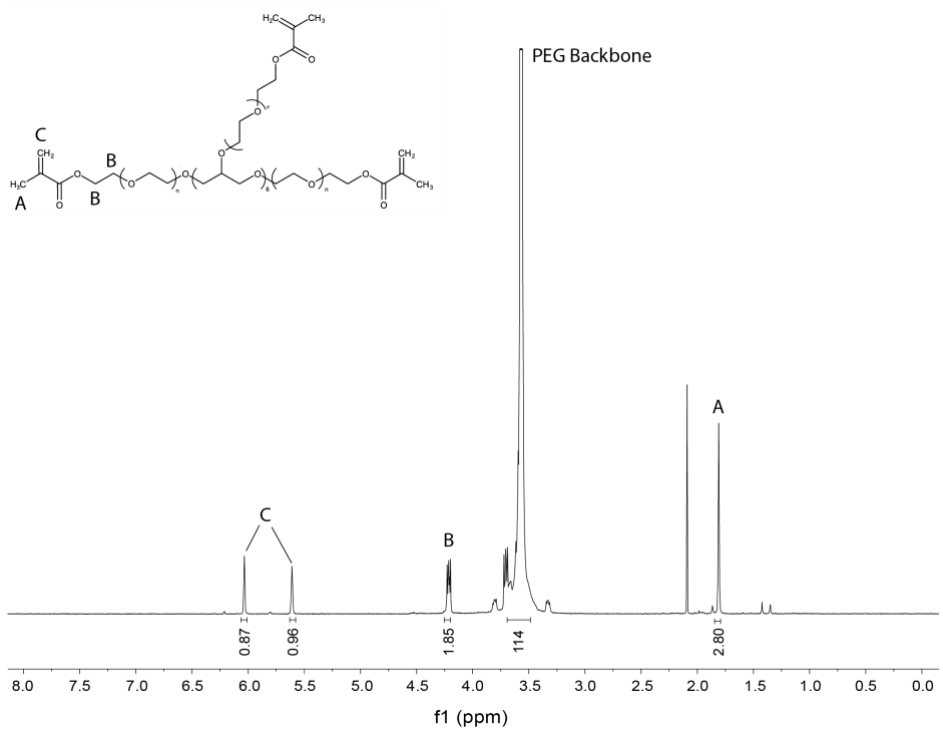

Fig. S3 H_1 NMR spectrum of PEGMA ($CDCl_3$, 300 MHz). The degree of vinyl end group, $C=CH_2$, calculated by the integration ratio of peak C (2H), compared to the peak of the PEG backbone was found to be 91%. $_1H$ NMR (300 MHz, $CDCl_3$): δ (ppm) 1.23 (t, 6H, CH_3–), 3.62 (s, 114H, PEG backbone), 4.17-4.21 (t, s, 8H, $–CH_2–C(O)–O–O$, $–O–CH_2–C(=CH_2)–$), 5.90 (s, 1H, $–C=CH_2$), 6.31 (s, 1H, $–C=CH_2$).

Confirmation of decellularization: Sufficient decellularization was confirmed through quantification of dsDNA by Quant-iT™ PicoGreen™ dsDNA Assay Kit (ThermoFisher Scientific), analysis of residual DNA fragments by gel electrophoresis, and hematoxylin and eosin staining of the decellularized lung tissue as previously described.[3] Results of all three analysis techniques showed sufficient decellularization compared to the criteria recommended by Badylak and colleagues (Fig. S4).[4]

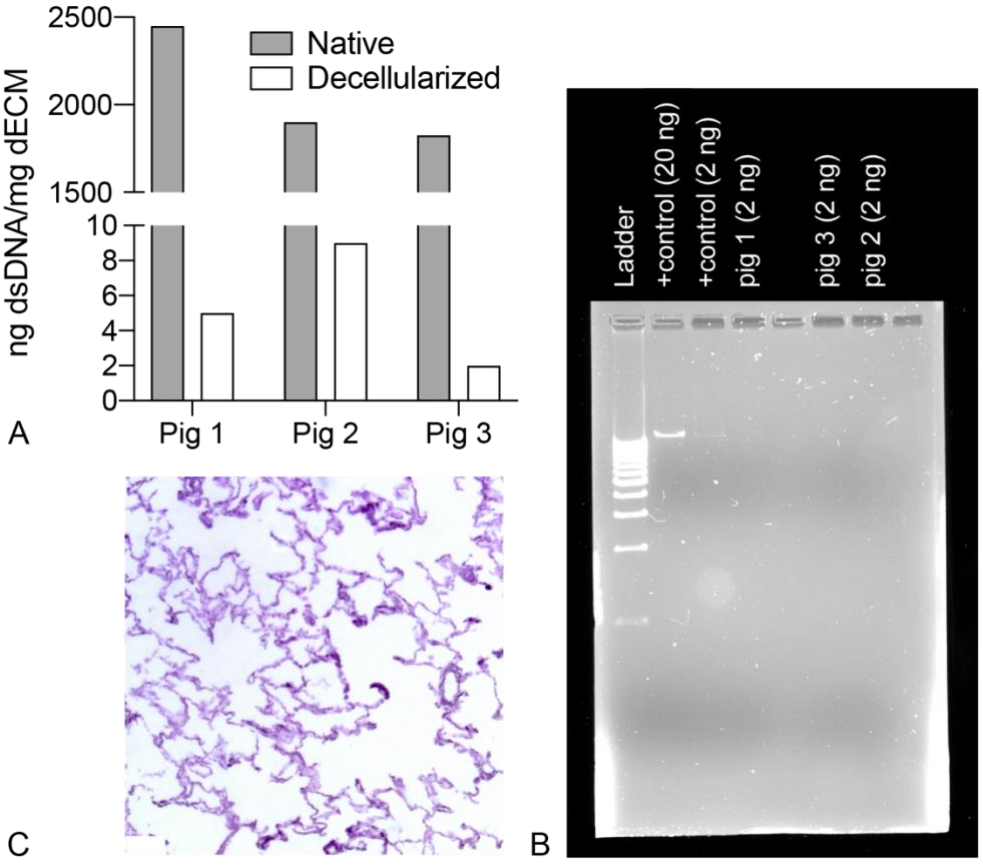

Fig. S4 A) dsDNA content was significantly reduced following the decellularization protocol to below the recommended minimal criteria of 50 ng dsDNA per mg dECM dry weight. B) There were no DNA fragment lengths above 200 bp as measured by gel electrophoresis following decellularization. C) There was a lack of visible nuclear material in tissue sections stained with hematoxylin and eosin. Scale bar, 2 cm.

Equilibrium swelling: Hybrid-hydrogel network formation was further characterized by measuring and calculating the experimental volumetric swelling ratio. Soft and stiffened hybrid-hydrogels were allowed to swell in phosphate buffered saline (PBS) and the swollen mass of n=4 replicates was measured at 1, 2, 6, 24, and 48-hour time points. The hydrogels were then placed in deionized water and lyophilized in order to record the dry polymer mass. The volumetric swelling ratio (Q) was calculated using equation (1)

$$Q = 1 + \frac{\rho_{PEG}}{\rho_{solvent}}\left(\frac{M_s}{M_d} - 1\right) \quad (1)$$

where ρ_{PEG} is the polymer density, $\rho_{solvent}$ is the solvent density, M_s is the swollen mass of the hydrogel and M_d is the dry mass.[5] The experimental equilibrium swelling ratio for the soft hybrid hydrogel reached 23.08 ± .36 within 6 h and the stiffened hybrid hydrogel reached 11.77 ± 0.37 within 6 h (Fig. S5). The experimental swelling ratio for the soft hydrogel was approximately twice the experimental equilibrium swelling ratio of the stiffened hydrogel, an indication that the secondary crosslinking reaction is in fact increasing overall crosslinking density after stiffening.[6]

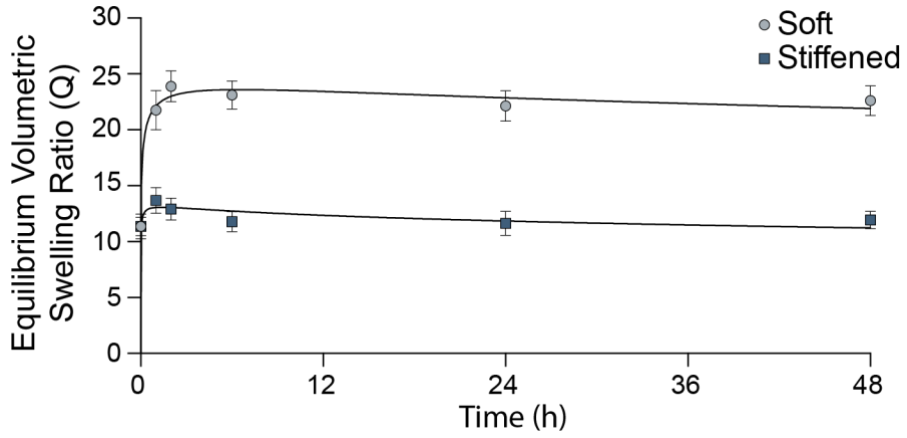

Fig. S5 Experimental equilibrium volumetric swelling ratio (Q) calculations revealed that both soft and stiffened hybrid-hydrogels reached an equilibrium value within 6 h of swelling in PBS. The equilibrium volumetric swelling ratio of the soft hybrid-hydrogel was approximately two times higher than the stiffened hybrid-hydrogel indicating differences in crosslinking density.

Cell activation. Fully synthetic hydrogels were formulated with 10 wt% PEGαMA crosslinked with 100% DTT and 1 mM CGRGDS pendant peptides. PDGFRα+ dual-reporter fibroblasts were seeded onto soft and stiff synthetic hydrogels. On day 6 LAP photoinitiator was added to the culture media on half of the soft samples, and 365 nm UV light at 10 mW/cm$_2$ was applied for 5 min at day 7 to stiffen these substrates. Representative images show that these PDGFRα+ dual-reporter fibroblasts exhibited a phenotypic transition into activated myofibroblasts through morphological changes, such as increased spreading and increases in expression of Col1a1 and αSMA in the PDGFRα+ dual-reporter fibroblasts that were cultured on stiff and stiffened synthetic hydrogels compared to those cultured on soft synthetic hydrogels.

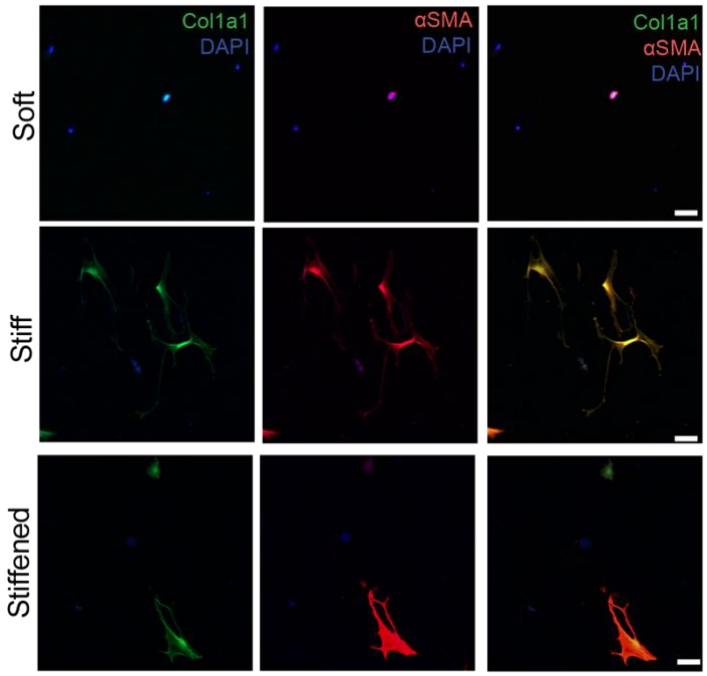

Fig S6. Representative images of dual-reporter fibroblasts on soft and stiff synthetic hydrogels on day 7 and stiffened hybrid hydrogels on day 9 showing expression of Col1a1-GFP and αSMA-RFP. Dual-reporter fibroblasts show morphological changes, such as increased spreading, on the stiff and stiffened hybrid-hydrogels that are absent in the fibroblasts cultured on soft hydrogels. Scale bars, 25 μm.

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