Supporting Information

Mimicking Physiologically Relevant Hepatocyte Zonation Using Immunomodulatory Silk Liver Extracellular Matrix Scaffolds toward a Bioartificial Liver Platform

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Table S1: Forward and reverse primer sequence for real-time polymer chain reaction (RT-PCR)

S. No.	Gene Name	Accession Number	Sequence
1.	r-GAPDH	XM_032905640.1	**F** 5'-TGACTCTACCCACGGCAAGTTCAA-3'
			R 5'-ACGACATACTCAGCACCAGCATCA-3'
2.	r-ALB	XM_032916218.1	**F** 5'-GATGCCGTGAAAGAGAAAGC-3'
			R 5'-CGTGACAGCACTCCTTGTTG-3'
3.	r-FIB	XM_032901307.1	**F** 5'-GGATCCCCTCCCAGAGAAGT-3'
			R 5'-GGGTGTGGAAGGGTAACCAG-3'
4.	r-GSTA1	XM_032909659.1	**F** 5'-ATGAGAAGTTTATACAAAGTCC-3'
			R 5'-GATCTAAAATGCCTTCGGTG-3'
5.	r-CYP1A2	XM_032911352.1	**F** 5'-CGGTGGCTAATGTCATCGGAG-3'
			R 5'-TTGCTGCTCTTCACGAGGTTGA-3'
6.	4-A1AT	XM_032908315.1	**F** 5'-AACAATGGGGCTGACCTC-3'
			R 5'-CCACAAAGATGGGGCTCT-3'
7.	r-CYP2E1	XM_032891525.1	**F** 5'-TGCGGAGGTTTTCCCTAAGC-3'
			R 5'-GCGCAGCCAATCAGAAATGT-3'
8.	r-HNF4α	XM_032904886.1	**F** 5'-AGTGCTGCCTTGGACCCAGCCT-3'
			R 5'-GGCACACAGGGCACTGACACCC-3'
9.	r-CK-19	XM_032913310.1	**F** 5'-CTAATGGCGAGCTGGAGGTGAAG-3'
			R 5'-GGCGGGCATTGTCGATCTGTAGGA-3'
10.	r-Sox9	XM_032913232.1	**F** 5'-TGGCAGAGGGTGGCAGACAGC-3'
			R 5'-CGTTGGGCGGCAGGTATTGG-3'

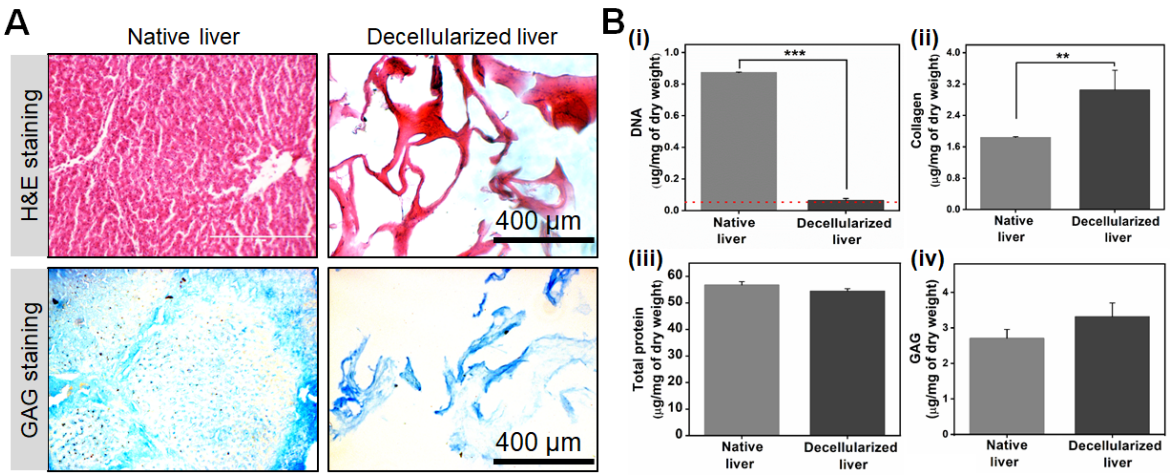

Figure S1: Decellularization of porcine liver and its characterization. A. *H & E and Alcian blue stained images of native and decellularized porcine liver tissues. Scale bar: 400 µm.* ***B.*** *Biochemical characterization of native and decellularized liver* ***(i)*** *DNA content,* ***(ii)*** *Collagen content,* ***(iii)*** *Total protein content, and* ***(iv)*** *GAG content. Data are expressed as µg per mg of dry weight of the tissue (n=9). The red dashed line in DNA content represents the reported permissible limit of DNA (50 ng) in decellularized ECM. ** and *** signify the statistical difference between the groups at $p \leq 0.01$ and $p \leq 0.001$, respectively.*

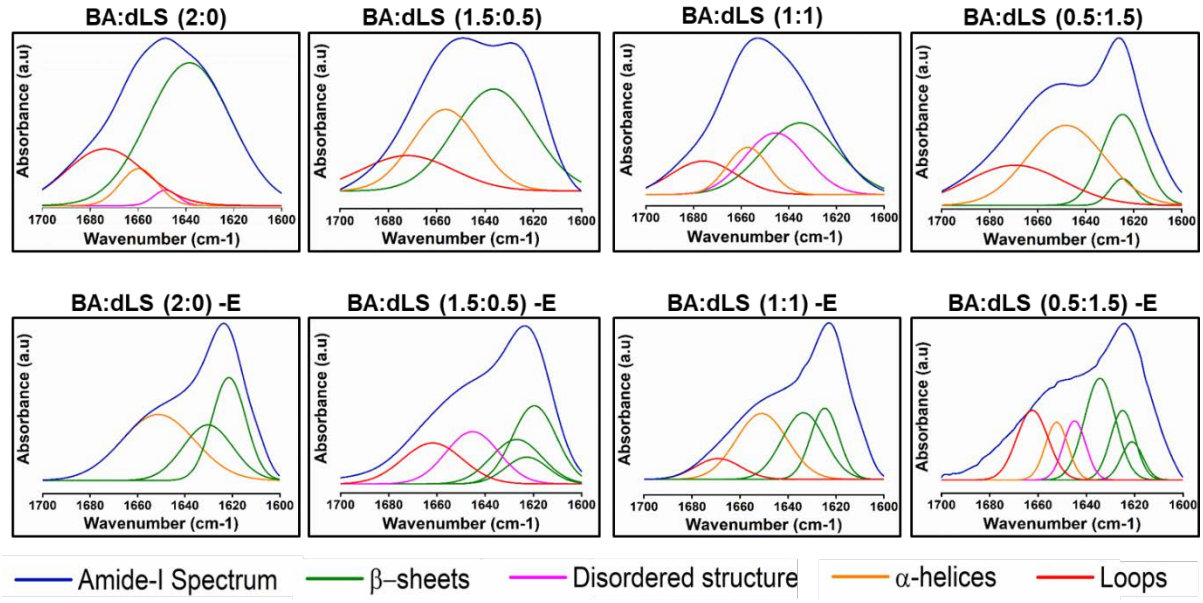

Figure S2: *Deconvolution of amide-I peak of untreated LECM-SF scaffolds (BA:dLS (2:0), BA:dLS (1.5:0.5), BA:dLS (1:1), BA:dLS (0.5:1.5)) and EDC/NHS crosslinked scaffolds (BA:dLS (2:0) -E, BA:dLS (1.5:0.5) -E, BA:dLS (1:1) -E, BA:dLS (0.5:1.5) –E) indicating conformational changes in secondary structure.*

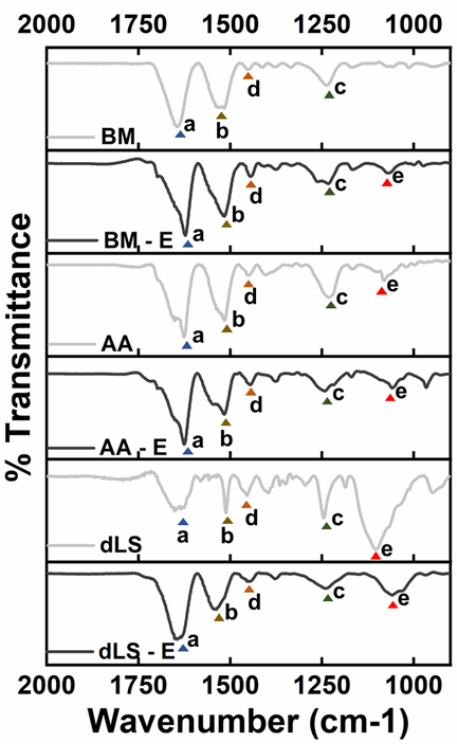

Figure S3: *FTIR spectrum of BM silk, AA silk and isolated dLS before (BM, AA, dLS) and after (BM -E, AA -E, dLS –E) EDC-NHS crosslinking.*

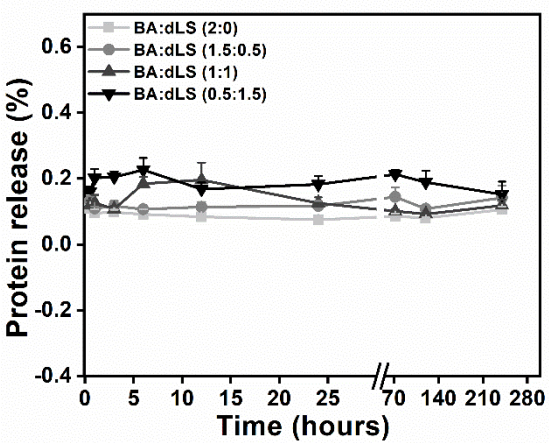

Figure S4: *Protein release from LECM-SF scaffolds over 10 days. Data are represented as average ± standard error mean (n=6).*

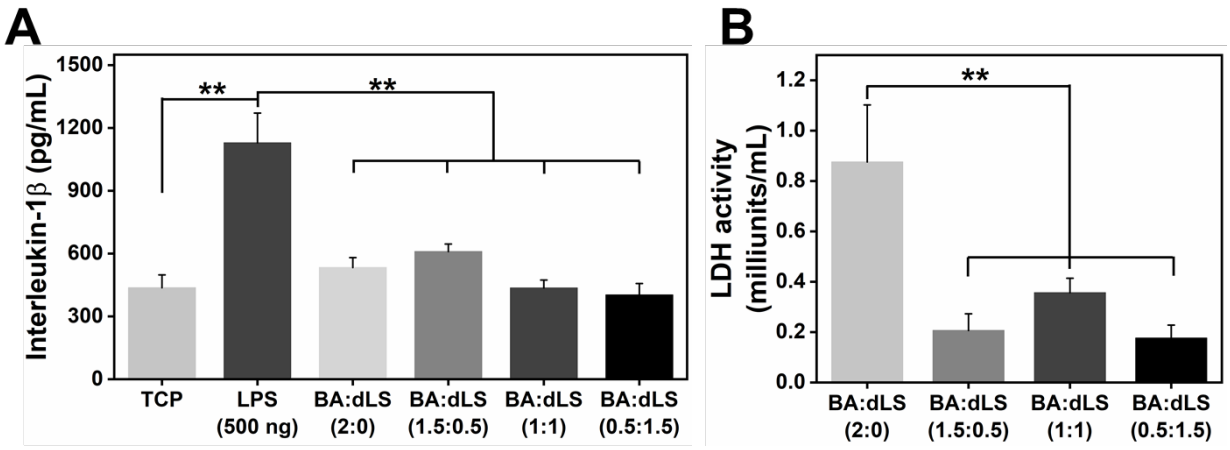

***Figure S5:* A.** *Assessment of in vitro immune response towards liver ECM blend silk scaffolds using RAW macrophages by measuring the secreted interleukin-1β.* **B.** *In vitro hemocompatibility towards liver ECM blend silk scaffolds. Data are represented as average ± standard error mean (n=4). ** signifies the statistical difference between the groups at $p \leq 0.01$.*

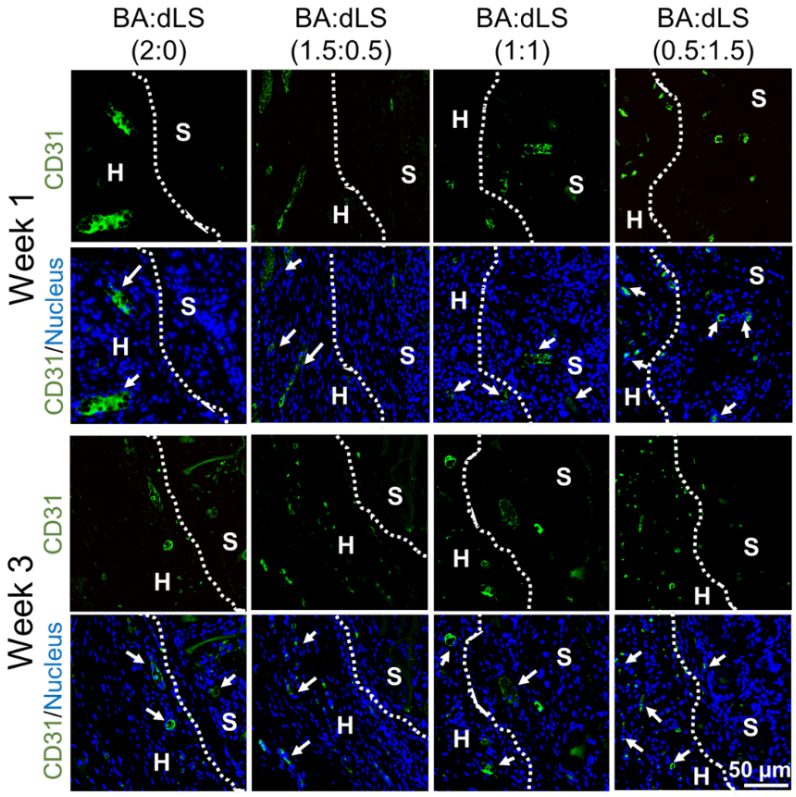

Figure S6: *CD31 staining images of explants from week 1 and week 3 postimplantation. The white dashed line demarcates the host tissue (H) and scaffold (S). The white arrows indicate the presence of blood vessels. Scale bar: 50 μm.*

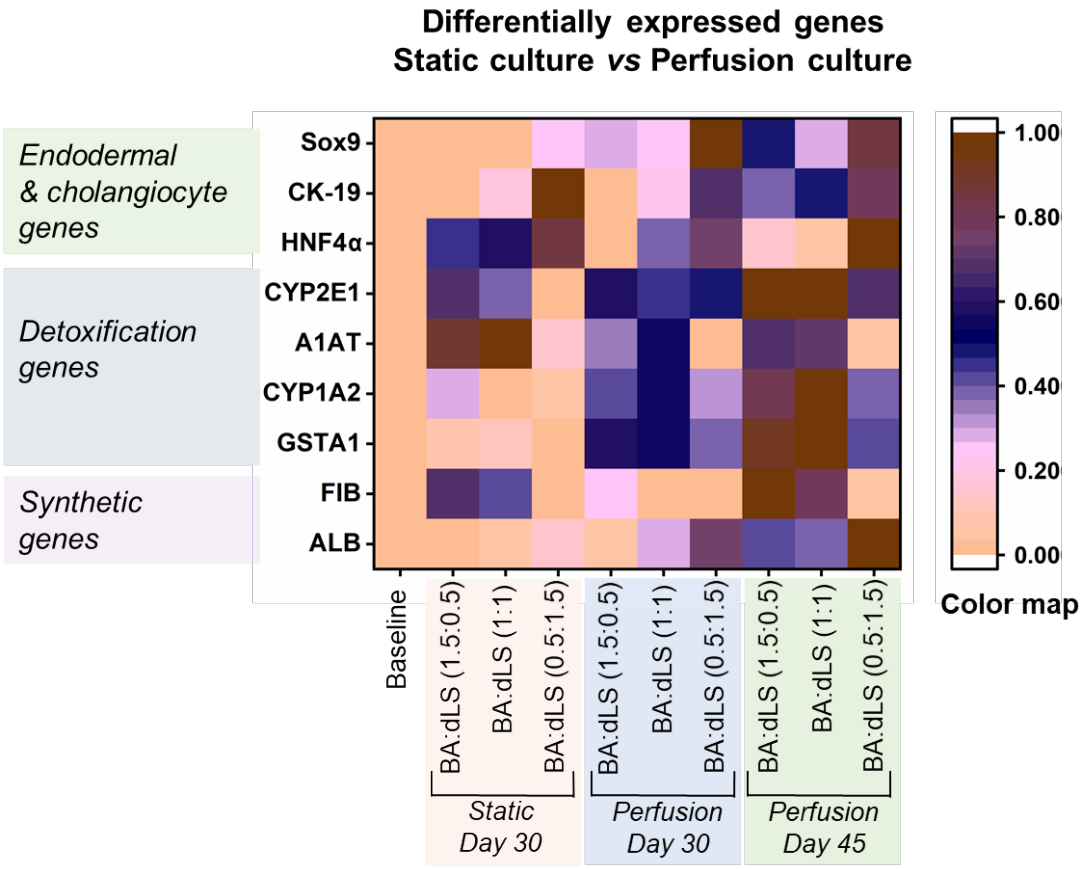

Figure S7: *Heat map showing the relative expression levels of synthetic genes (albumin-ALB, fibronectin-FIB), detoxification genes (glutathione S-transferase alpha 1-GSTA1, cytochrome P450 1A2-CYP1A2, alpha-1 antitrypsin-A1AT, cytochrome P450 2E1-CYP2E1), endodermal & cholangiocyte genes (hepatocyte nuclear factor 4α-HNF4α, cytokeratin 19-CK19, SRY-box transcription factor9-Sox9) in PNRHs cultured on LECM-SF scaffolds during static (day 30) versus perfusion bioreactor conditions (day 30 and day 45).*