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Supplementary information to:

Original article:

BIOENGINEERING SCALABLE AND DRUG-RESPONSIVE *IN VITRO* HUMAN MULTICELLULAR NON-ALCOHOLIC FATTY LIVER DISEASE MICROTISSUES ENCAPSULATED IN THE LIVER EXTRACELLULAR MATRIX-DERIVED HYDROGEL

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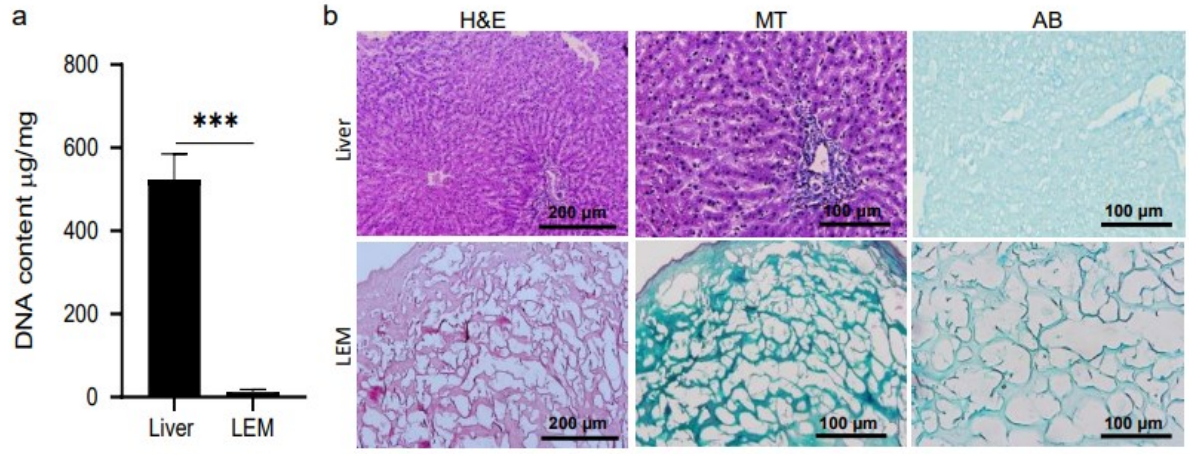
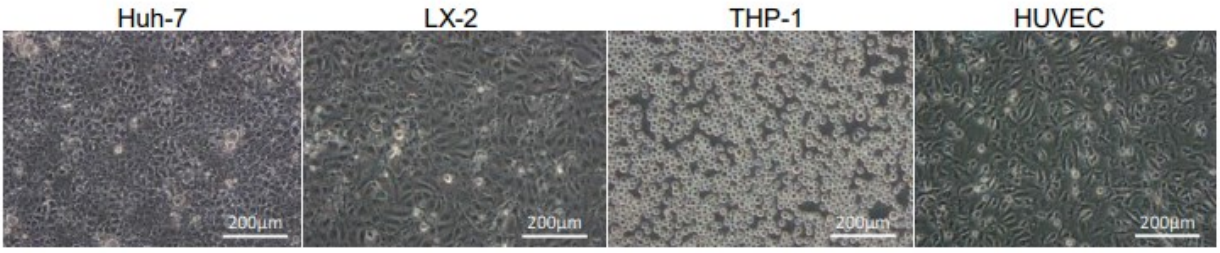


Supplementary Figure 1: Characterization of the decellularized liver-derived extracellular matrix (LEM). **a)** Quantification of DNA content in the liver and LEM. **b)** Histological evaluations of the liver and the resultant LEM. Hematoxylin and eosin (H&E) staining showed that, compared to the native liver, there are no nuclei in the LEM. Preservation of collagen and glycosaminoglycans (GAGs) in the LEM was demonstrated using Masson's trichrome (MT) and Alcian blue (AB) staining, respectively.



Supplementary Figure 2: Phase-contrast micrographs of Huh7, LX-2, and THP-1 cell lines and HUVEC, demonstrated the cells morphology in two-dimensional culture condition.

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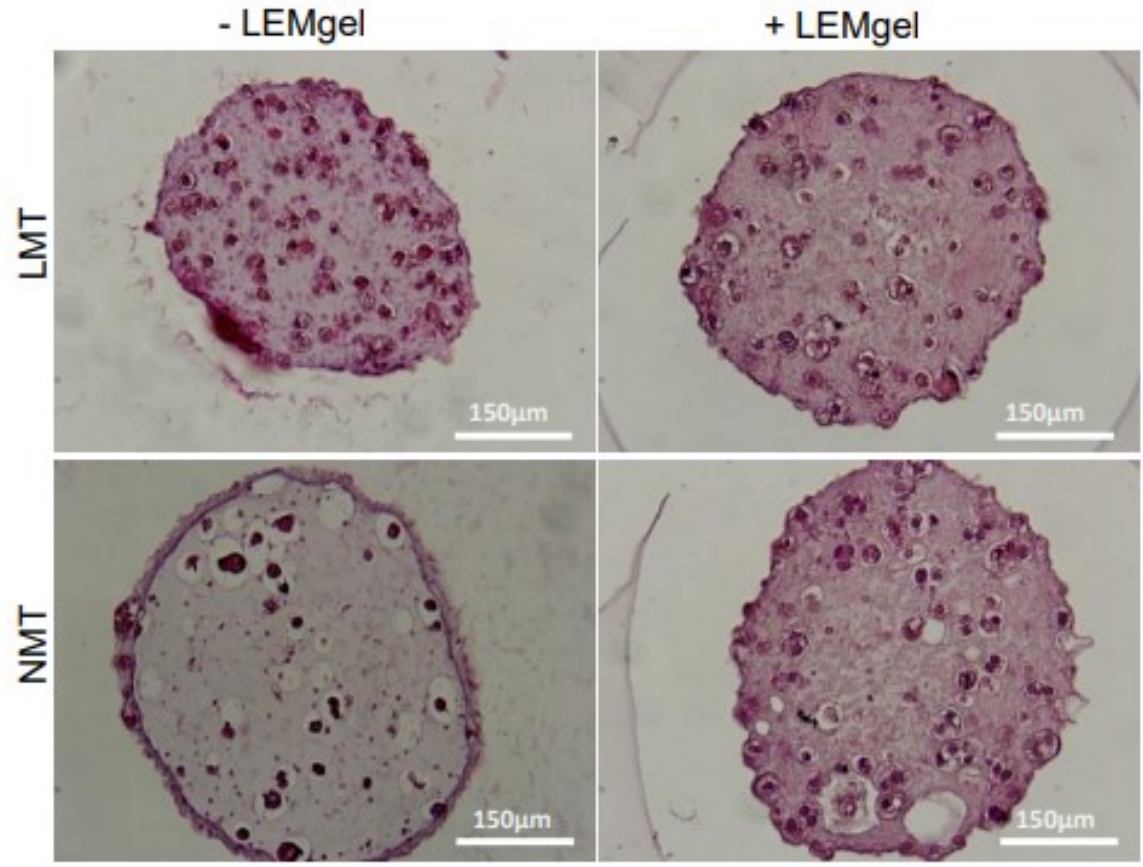

Supplementary Figure 3: Light micrographs from Hematoxylin and eosin-stained sections of the LMTs and NMTs generated in the presence or absence of LEMgel on day 8. As it is visible, the overall structures of the +LEMgel microtissues are more appropriate with homogenous distribution of live cells. While the –LEMgel microtissues revealed visible destruction in their structure and there are many necrotic cells with pyknotic nuclei, particularly in the NMT.

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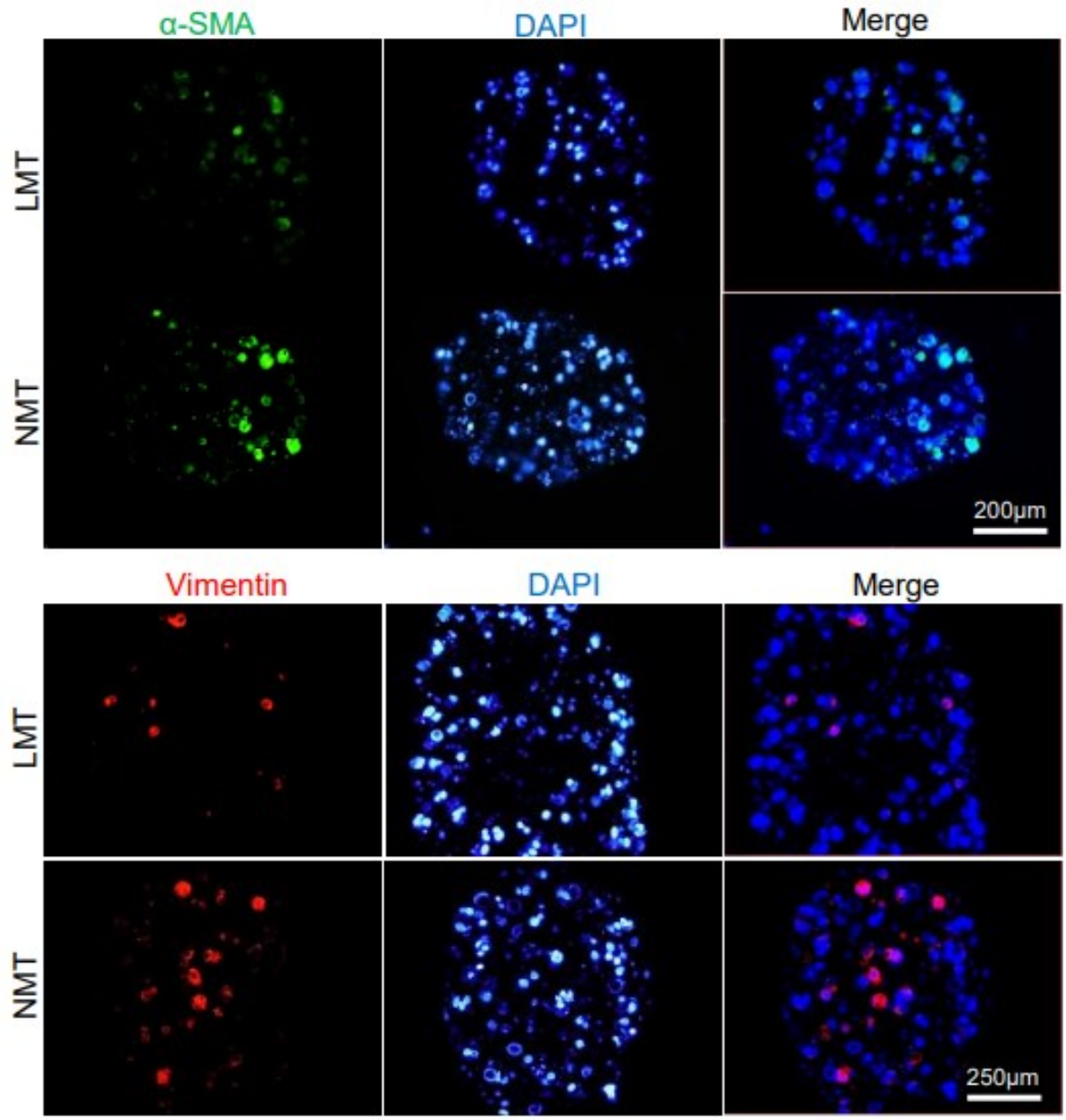

Supplementary Figure 4: Representative immunofluorescence micrographs from the microtissue sections stained against Vimentin and α-SMA on day 8. The micrographs revealed that there are more Vimentin-positive and α-SMA-positive cells in the NMTs in comparison to the LMTs.

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Supplementary Table 1: Primer sequences used in qRT-PCR

Genes	Sequence
CPT1	F: ATCAATCGGACTCTGGAAACGG R: TCAGGGAGTAGCGCATGGT
CD36	F: CTTTGGCTTAATGAGACTGGGAC R: GCAACAAACATCACCACACCA
SREBP	F: CGGAACCATCTTGGCAACAGT R: CGCTTCTCAATGGCGTTGT
CYP2E1	F: GTGATGCACGGCTACAAGG R: GGGTGGTCAGGGAAAACCG
IL 6	F: AGGAGACTTGCCTGGTGAAA R: CAGGGGTGGTTATTGCATCT
IL 8	F: TAGCAAAATTGAGGCCAAGG R: AGCAGACTAGGGTTGCCAGA
IL 10	F: AAGCTGAGAACCAAGACCCA R: AAGGCATTCTTCACCTGCTC
TGF-β	F: GAAACCCACAACGAAATCTATGA R: TAACTTGAGCCTCAGCAGAC
TNF-α	F: CCTCTCTCTAATCAGCCCTCTG R: GAGGACCTGGGAGTAGATGAG
MMP-2	F: ATTGTATTTGATGGCATCGCTC R: ATTCATTCCCTGCAAAGAACAC
COL1A1	F: ATGCCTGGTGAACGTGGT R: AGGAGAGCCATCAGCACCT
PCK1	F: GGCTGAAGAAGTATGACAACTG R: AAATCCTCCTCTGACATCCA
G6P	F: AACATCGCCTGCGTTATCCTC R: ACGTCCCGGATGATCCCAA
GAPDH	F: GAAATCCCATCACCATCTTCC R: GGCTGTTGTCATACTTCTCAT