Supplementary Information

A microfluidic patterned model of non-alcoholic fatty liver disease: applications to disease progression and zonation

Beyza Bulutoglu[1], Camilo Rey-Bedón[1], Young Bok (Abraham) Kang[1,†], Safak Mert[1], Martin L. Yarmush[1,2], O. Berk Usta[1,*]

[1] Center for Engineering in Medicine, Massachusetts General Hospital, Harvard Medical School and Shriners Hospitals for Children, Boston, MA, 02114, USA

[2] Department of Biomedical Engineering, Rutgers University, Piscataway, NJ, 08854, USA

[†] Current address: College of Engineering, George Fox University, Newberg, OR, 97132 USA

[*] Corresponding author, e-mail: berkusta@gmail.com; ousta@mgh.harvard.edu

Supplementary Figures

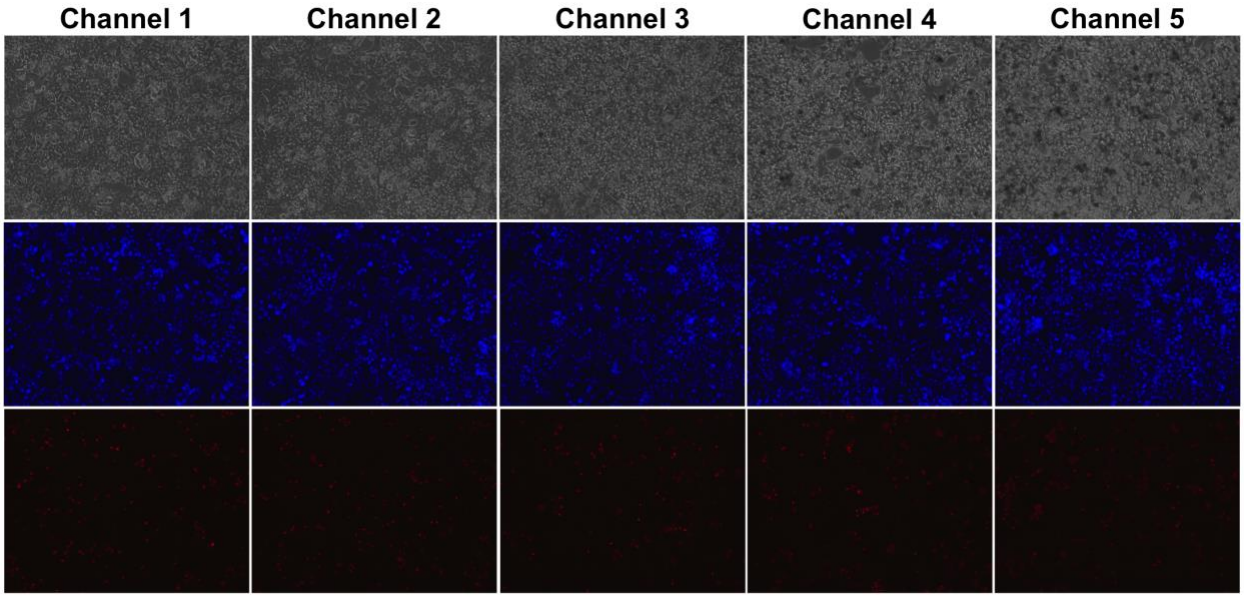

Figure S1. Representative channel pictures of live/dead staining. Following perfusion with a linoleic acid gradient of 0 - 2 mM for 24 hours, the primary rat hepatocytes were stained with Hoechst 33342 and ethidium homodimer-1 (EthD-1). No significant change in cell viability was detected across the device chamber. Top, middle, and bottom panels show the bright-field, Hoechst 33342, and EthD-1 pictures, respectively.

ACACA	2	3	4	5
1	ns	ns	*	**
2		ns	ns	ns
3			ns	ns
4				ns

ACSL4	2	3	4	5
1	ns	ns	*	***
2		ns	ns	***
3			ns	**
4				ns

ChREBP	2	3	4	5
1	ns	ns	ns	**
2		ns	ns	**
3			ns	**
4				*

EVOLV6	2	3	4	5
1	ns	ns	**	**
2		ns	*	**
3			*	*
4				ns

FASN	2	3	4	5
1	ns	ns	ns	**
2		ns	ns	*
3			ns	ns
4				ns

FATP5	2	3	4	5
1	ns	ns	ns	*
2		ns	ns	ns
3			ns	ns
4				ns

GCK2/3	2	3	4	5
1	ns	ns	**	***
2		ns	*	***
3			ns	**
4				ns

MRP3	2	3	4	5
1	ns	ns	ns	**
2		ns	ns	**
3			ns	**
4				ns

Figure S2. Statistical significance comparison of fold gene expression changes of hepatocytes in device channels. One-way ANOVA analysis was performed on quantitative real-time PCR results for each gene where the channels of the microfluidic device was compared to each other. ns = non-significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.005$.

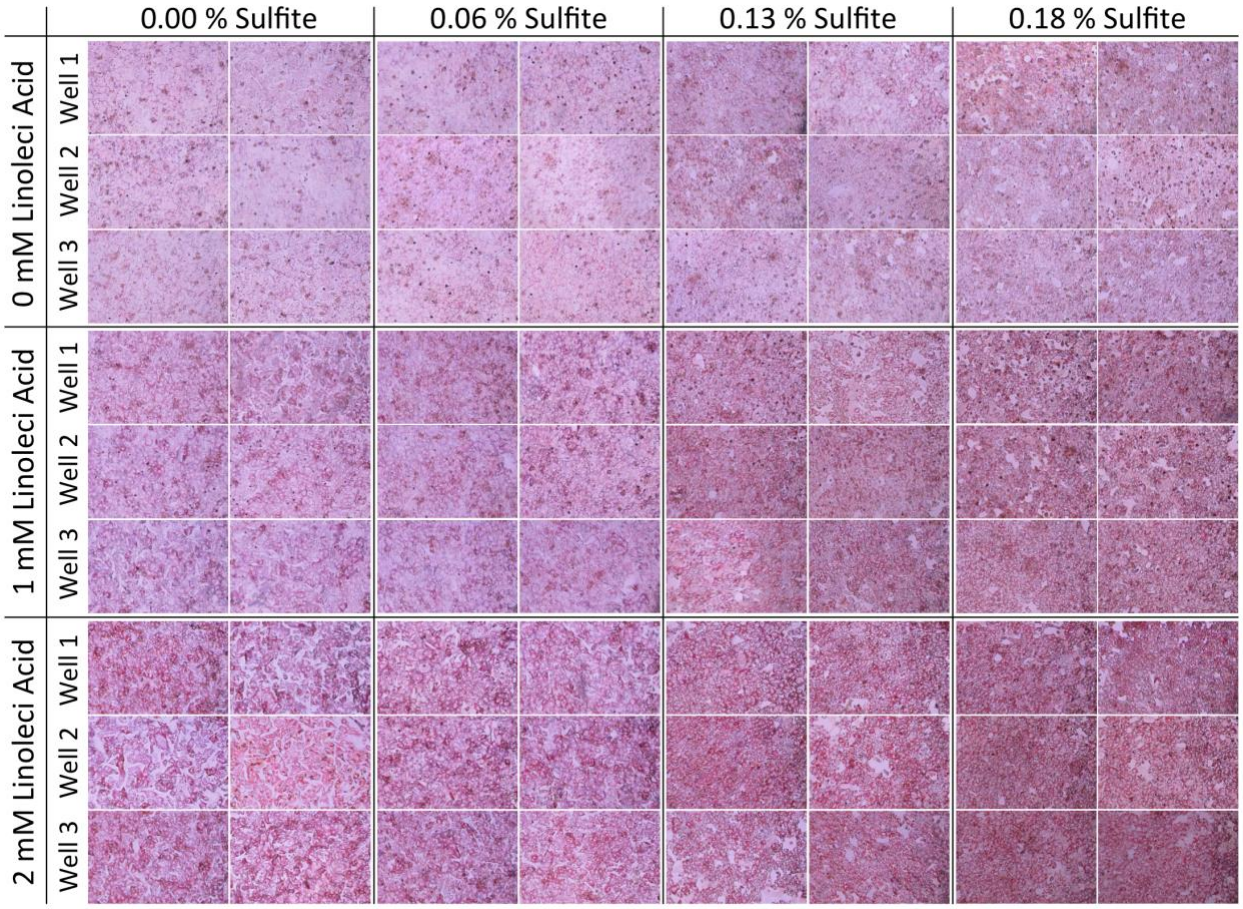

Figure S3. Oil-Red-O staining of primary rat hepatocytes cultured in well-plates. Primary hepatocytes were subjected to varying levels of sulfite/cobalt concentrations (0.00% sulfite & 0 µM cobalt - 0.18% sulfite & 18 µM cobalt) in the presence of varying linoleic acid (LA) concentrations (0 - 2 mM). A gradual increase in Oil-Red-O stain across oxygen gradient (deprivation) as well as free fatty acid gradient demonstrates the effect of both components on lipid accumulation in hepatocytes, where most fat storage was observed under extreme conditions: 2 mM LA & 0.18% sulfite/18 µM cobalt.

Supplementary Tables

SI Table 1. Sequences of qPCR Primers

Target	Forward sequence	Reverse sequence
ACACA	5’ ATTGGGGCTTACCTTGTCCG 3’	5’ GCACCGGCTCCTGTTAGAAT 3’
ACSL4	5’ AGTGGATAGCAGTTACAGTG GTG 3’	5’ GCGATATGGACTTCCGGGTTT 3’
ELOVL6	5’ CCGAAGATCAGCCCCAATGA 3’	5’ CGTACAGCGCAGAAAACAGG 3’
FATP5	5’ GAGTCCTCGGCTGCTTACAA 3’	5’ ACTCAGCCCAGTATCGGGAA 3’
FASN	5’ CCAAGTTCGACGCCTCCTTT 3’	5’ CCAGTGTTTGTTCCTCGGAGT 3’
GCK2/3	5’ ACGAGGAGGCCAGTGTAAAGAT 3’	5’ GTCTCCGACTTCTGAGCCTTC 3’
ChREBP	5’ CATCGATCCGACACTCACCC 3’	5’ TCCCGGCATAGCAACTTGAG 3’
MRP3	5’ GGGTACCATCCGTACCCAGT 3’	5’ CCTCCAGCTGCAATGAGGTT 3’