Supplementary Information

Microfibrous extracellular matrix changes the liver hepatocytes energy metabolism via integrins

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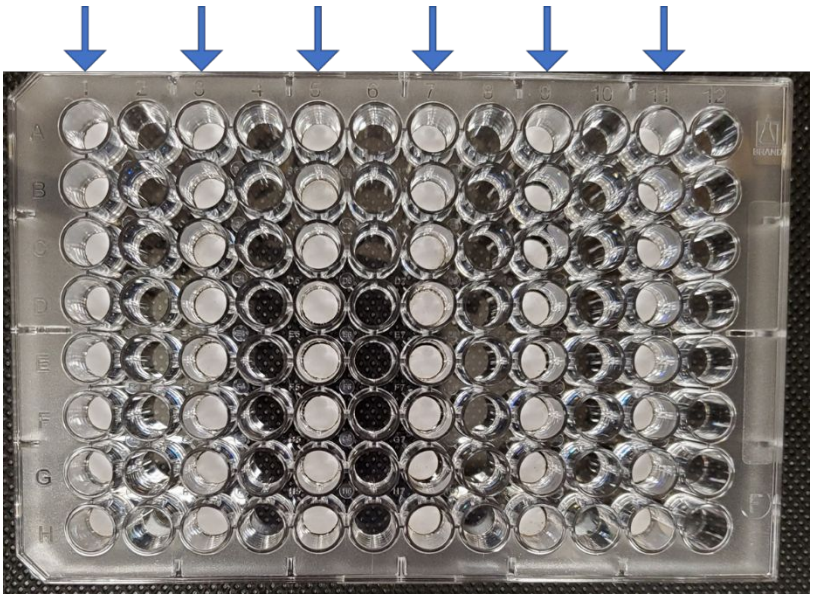

Figure S1. A 96-well plate with the fibrous scaffolds in alternating columns (arrows)

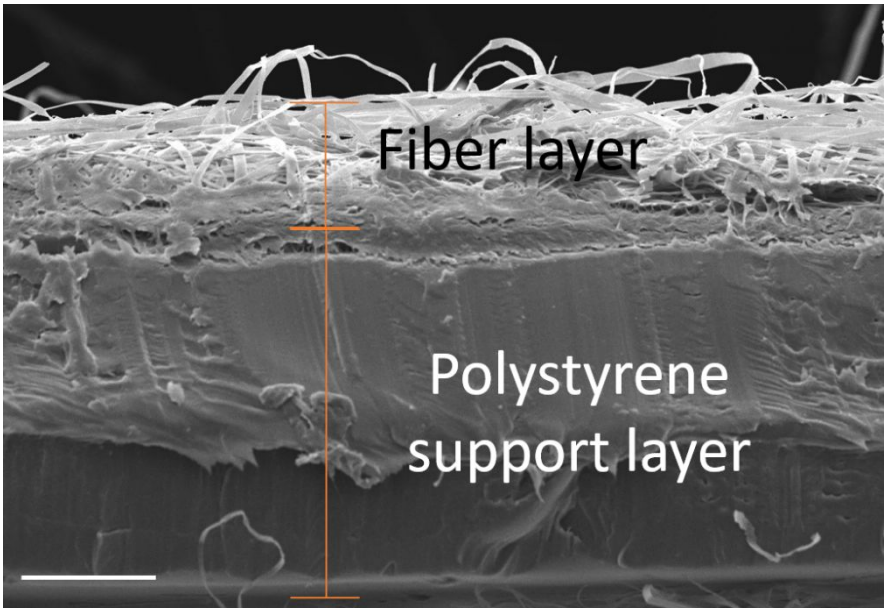

Figure S2. An SEM cross view of a fibrous insert. On the polystyrene support layer (250 μm), a layer of microfibers (50 μm) was deposited. Scale bar=50 μm.

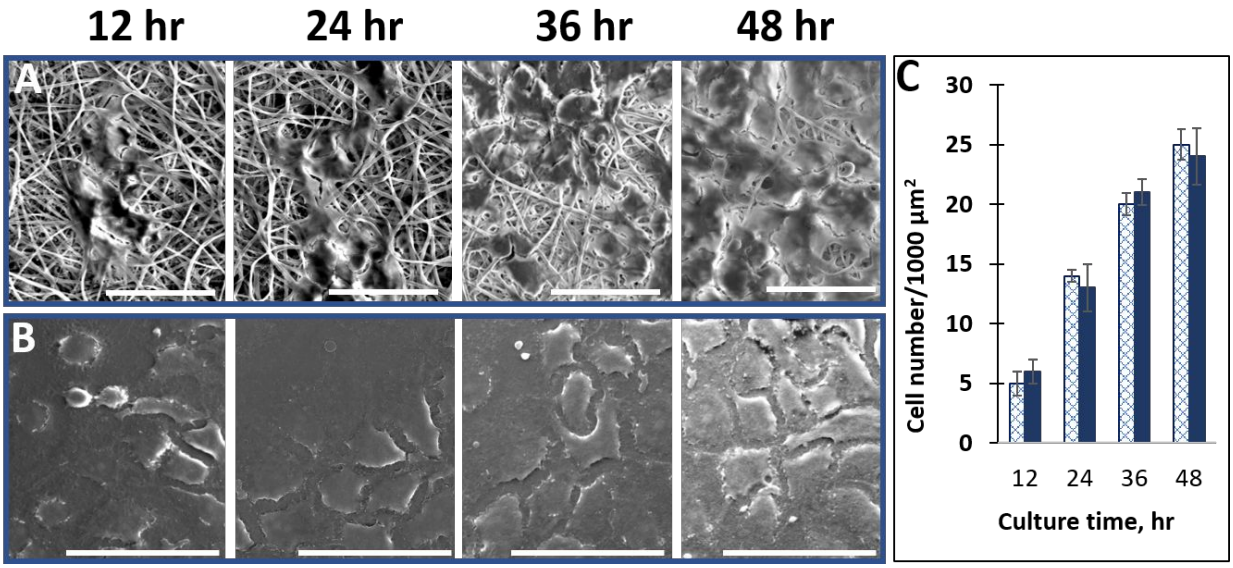

Figure S3. Characterization of Huh7 cell proliferation on the fibrous scaffold and the flat surface. **(A)** SEM images of the cells spreading on the fibers at different times after cell seeding. Scale bars=20 µm. **(B)** SEM images of the spreading cells on the flat surface. Scale bars=20 µm. **(C)** Quantitated cell coverage. After 48 hours, the cells on both substrates reached ~25 cells per 1000 μm^2 without significant differences. N=10, error=stdev.

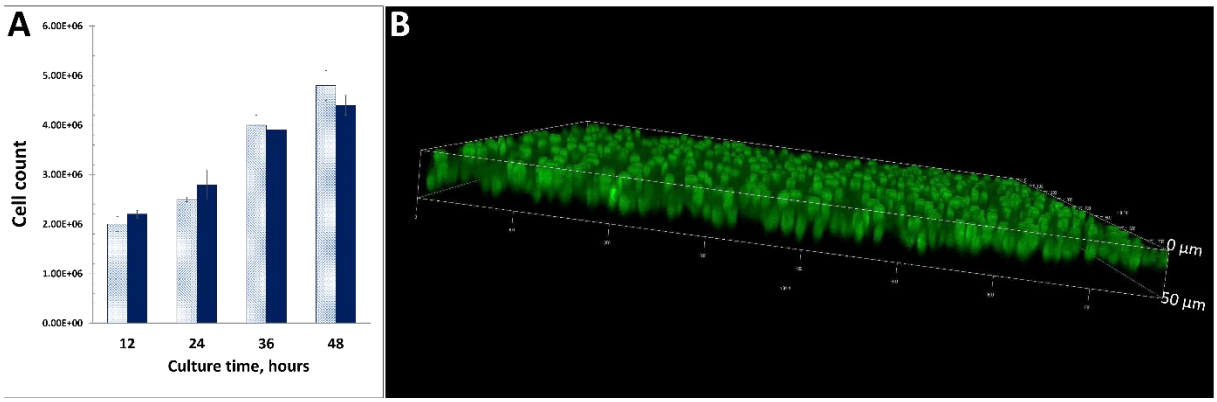

Figure S4. **(A)** Proliferation of Huh7 cells on the fibers and on flat (on fibers-mesh fill; on flat-solid fill). Cell suspensions with known cell counts (by a hemocytometer) were lysed in RIPA buffer, and the protein amounts were measured by the BCA assay to generate a calibration curve between protein amounts and cell counts. Cells cultured on the fibers and on flat were lysed under the same condition, and the protein concentrations in the lysates were used to calculate cell counts. N=20, error bar=S.E.M. **(B)** A confocal view of the cells distributing through the 50 µm thick fibrous scaffold. The cells were prelabeled by Calcein green. The average infiltration depth was determined to be 28 ± 5 µm. The cell infiltration into the fibrous pores was the reason why there were ~25% more cells on the fibrous scaffold than on flat.

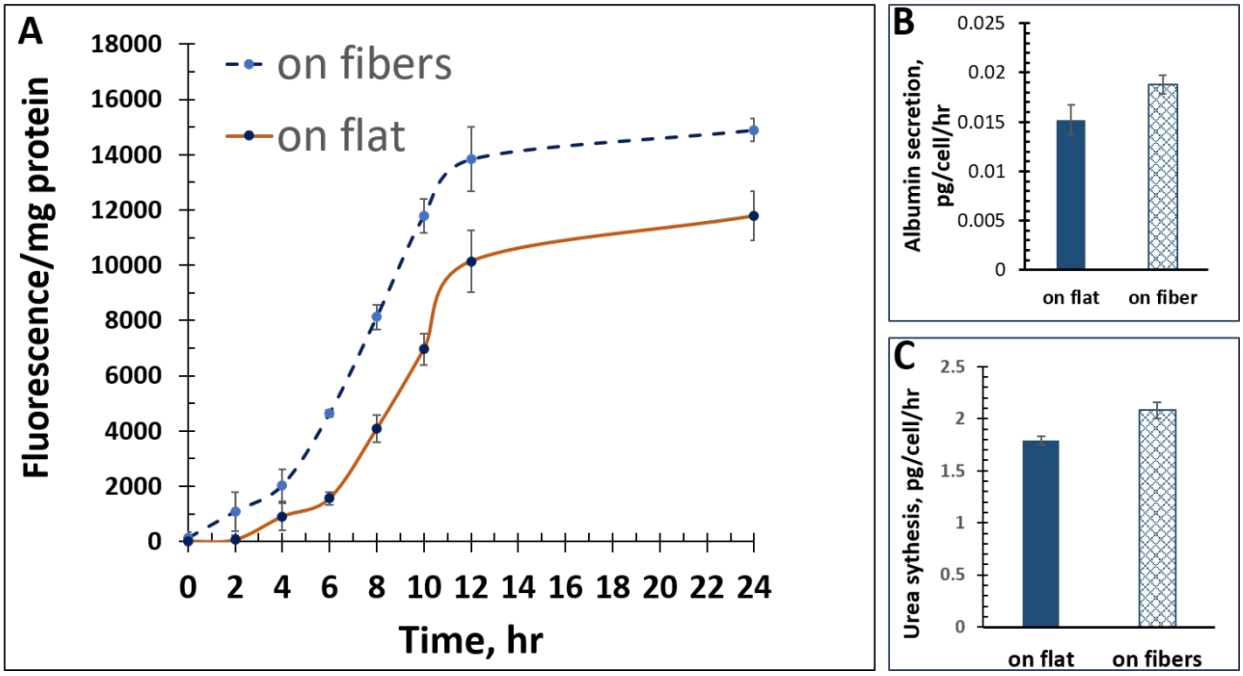

Figure S5. **(A)** Characterization of the CYP450 activity in Huh7s cultured on the fibers vs. on flat. The substrate 7-ethoxyresorufin was added to the culture media, which could be metabolized by CYP450 to fluorescent resorufin. After the 24-hour sampling, the cells on corresponding substrates were thoroughly rinsed with PBS, and completely lysed in a lysis buffer with ultrasonication bursting. The total amount of proteins in the lysates was used to normalize the detected fluorescent signals. Cells on the fibers showed higher CYP450 activities overall. N=5, error =stdev. **(B)** Measurements of secreted albumin from cells cultured on flat and on fibers. N=5, error=stdev. **(C)** Measured urea from the hepatocytes on the ECMs. N=5, error=stdev.