Electronic Supplementary Material (ESI) for Integrative Biology.


## Supplementary Figures

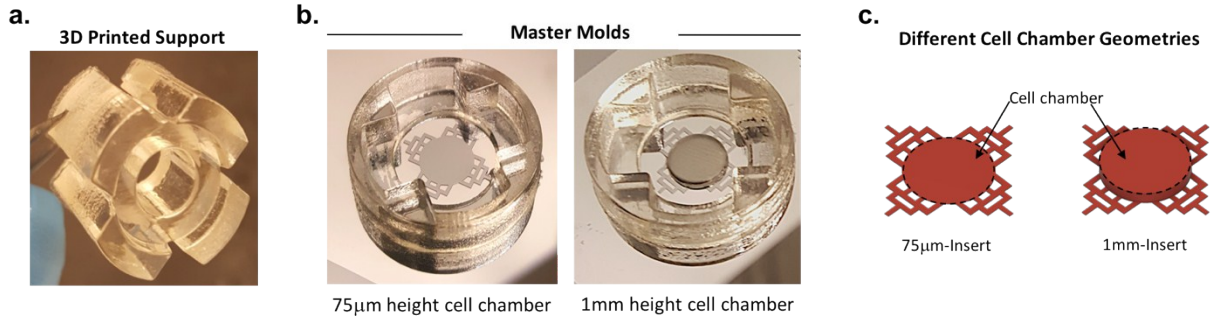


**Figure S1. (a)** 3D printed support structure used to fabricate the multiwell plate inserts. **(b)** Master molds used for fabricating both multiwell plate inserts (macro- and microscale cell culture chambers). **(c)** 3D schematic illustrating the difference in cell chamber geometries for the varied multiwell plate inserts.

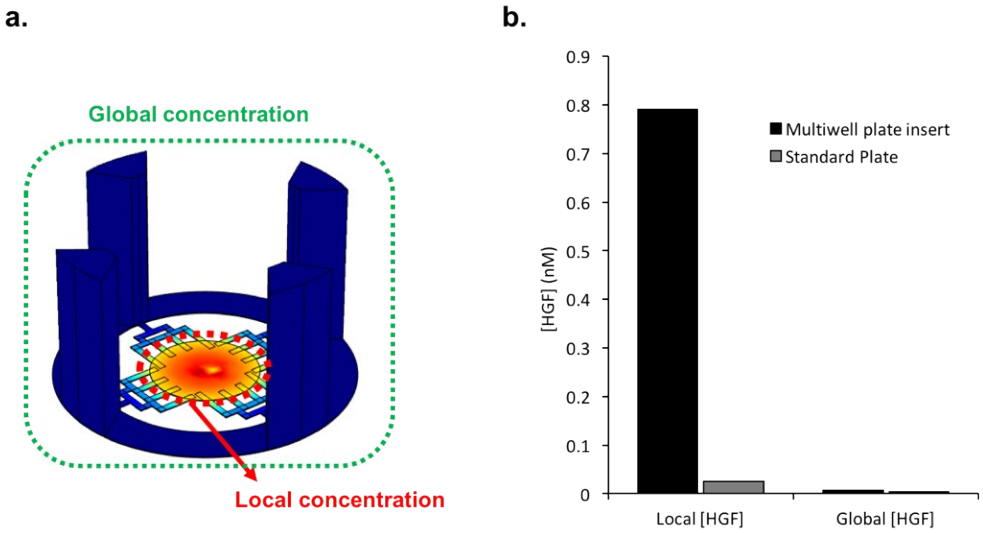


**Figure S2.** Numerical simulation for HGF accumulation. **(a)** Schematic defining global vs. local concentration. **(b)** COMSOL generated numerical simulation for local and global HGF concentration (nM) after 24 h of culture in multiwell plate insert vs. standard plate.

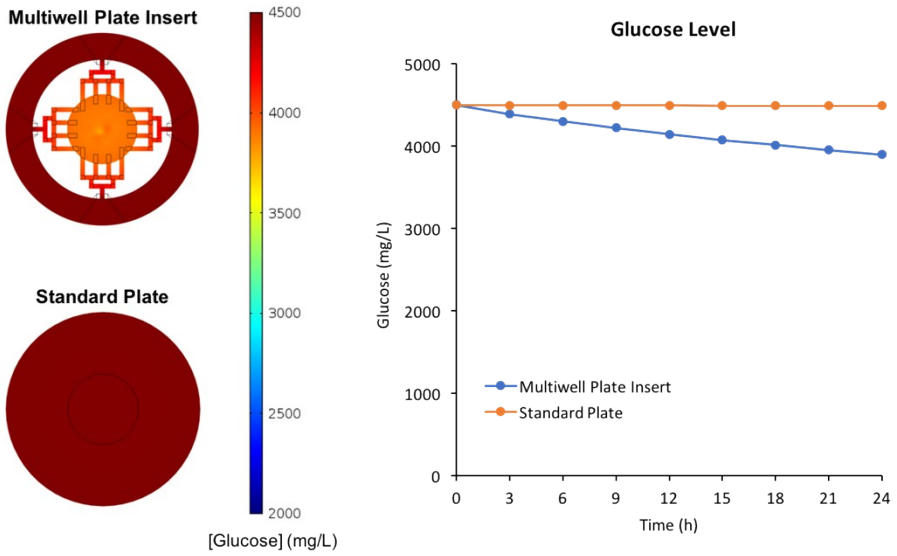


**Figure S3. Numerical simulation for glucose level.** COMSOL modeling of local glucose concentration in multiwell plate insert vs. standard plate over the course of 24 h.

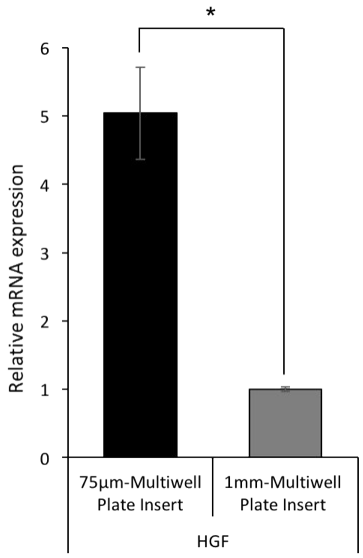


**Figure S4.** HGF relative expression levels for primary rat hepatocytes obtained through RT-qPCR on day 11 of culture. Data shown are mean ± standard deviation (n=2) for each sample type. *$p<0.05$.

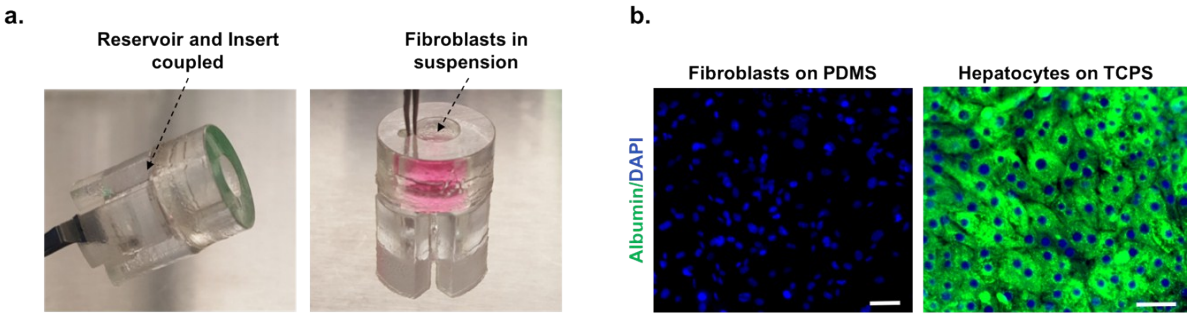


**Figure S5. (a)** Images of cell seeding reservoir coupled with the insert for the purposes of seeding fibroblasts on the PDMS roof of the insert. In the image on the right, cells in suspension are added into the reservoir. **(b)** Albumin immunostaining (green) of primary hepatocytes and fibroblasts in the co-culture setting. Nuclei were stained with DAPI (blue). Scale bar=50μm.

## Supplementary Table

**Table S1:** The primers used for RT-qPCR

| | |
|---|---|
| Albumin-F | CAT CCT GAA CCG TCT GTG TG |
| Albumin-R | TTT CCA CCA AGG ACC CAC TA |
| HGF-F | CTT CTG CCG GTC CTG TTG |
| HGF-R | TCT TCT CTT CTT CTG TCC TTC TGC |
| E-cadherin-F | CGT GGA TGT GGT AGA CGT GAA |
| E-cadherin-R | TTC TTC GCA GGC ACA AAA AT |
| CYP3A1-F | CTC CTC CTG CAG TTT CTT CTG TGT A |
| CYP3A1-R | CAG CAG CAC ACT TTC CTT TGT C |
| GAPDH-F | AGA CAG CCG CAT CTT CTT GT |
| GAPDH-R | CTT GCC GTG GGT AGA GTC AT |

**Table S2:** Molecular weight and diffusion coefficients of cell byproducts and growth factors

| **Molecule** | **Molecular weight (Da)** | **Diffusion Coefficient in water ($cm^2$ / s)** |
|---|---|---|
| HGF | 84,000 Da | $8.5 \times 10^{-7}$ [1] |
| $CO_2$ | 44 Da | $1.92 \times 10^{-5}$ [2] |
| Ammonia | 17 Da | $1.64 \times 10^{-5}$ [2] |
| Urea | 60 Da | $1.38 \times 10^{-5}$ [3] |
| TGFβ1 | 25,000 Da | $2.6 \times 10^{-7}$ [1] |

**References:**


1. Young, M.E., Carroad, P.A. & Bell, R.L. Estimation of Diffusion-Coefficients of Proteins. *Biotechnol Bioeng* **22**, 947-955 (1980).
2. Cussler, E.L. Diffusion, mass transfer in fluid systems. (Cambridge University Press, Cambridge Cambridgeshire ; New York; 1984).
3. Truskey, G.A., Yuan, F. & Katz, D.F. Transport phenomena in biological systems, Edn. 2nd. (Pearson Prentice Hall, Upper Saddle River, N.J.; 2009).