

Supplementary Materials

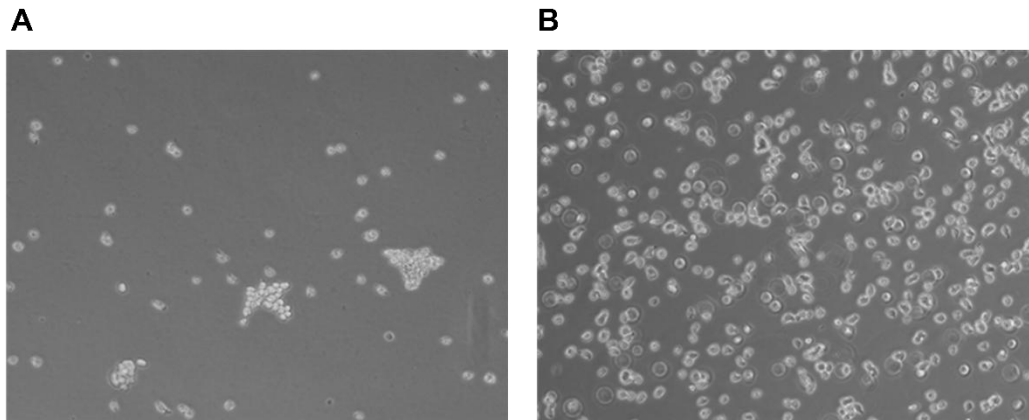


Fig. S1. Morphological observation of detached HLSCs cultured in M11 with 1.2% FCS following treatment with **A** TrypLE for 15 min and **B** Accutase for 5 min. HLSCs: human liver stem cells, FCS: fetal calf serum.

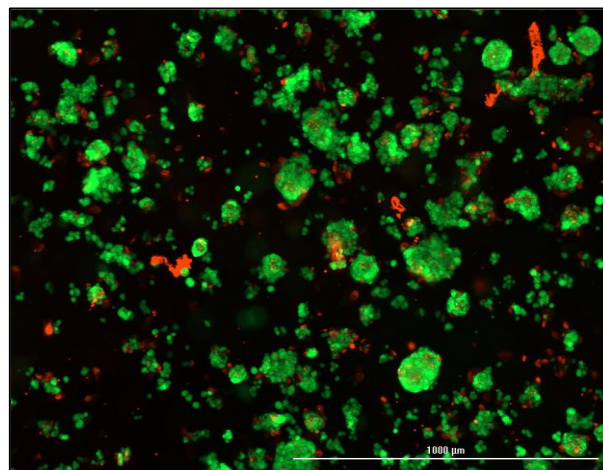


Fig. S2. Viability staining of ILS formed by HLSC expanded in 2-L STR for 120 h in M11 with 1.2% FCS on B2 microcarriers using FDA (green) and PI (red). The scale bar is 1,000 μm . ILS: islet-like structures, HLSC: human liver stem cell, STR: stirred tank bioreactor, FCS: fetal calf serum, FDA: fluorescein diacetate, PI: propidium iodide.

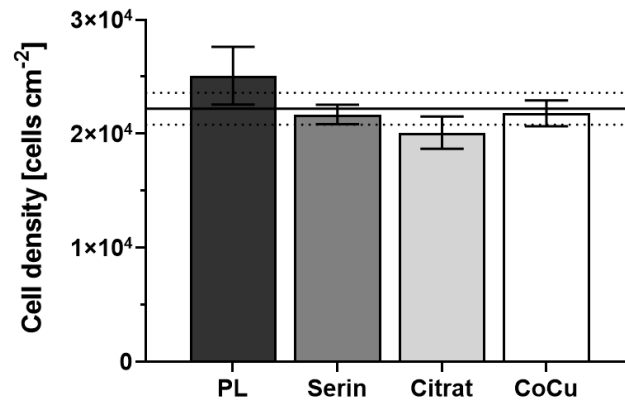


Fig. S3. HLSC expansion in passage six in M11 with 1.2% FCS on B2 surfaces after 120 h, supplemented with PL, serine, citrate, and CoCu. The solid line represents the mean, and the dotted line indicates the SD. Data are presented as mean $\pm$ SD ($n = 3$). HLSC: human liver stem cell, FCS: fetal calf serum, PL: phospholipids, CoCu: cobalt-copper.

Table S1. Working ranges of factors with a significant impact on HLSC growth, as determined using a OFAT model

Additive	Lower limit	Upper limit
HSA ($\mu\text{g mL}^{-1}$)	0.0	80.0
FCS <i>v/v</i> (%)	0.0	0.5
ITS (mg L^{-1})	0.0	4.0
FGF-1 (ng mL^{-1})	0.0	10.0
EGF/FGF-2 (ng mL^{-1})	0.0	10.0

HLSC: human liver stem cell, OFAT: one-factor-at-a-time, HSA: human serum albumin, FCS: fetal calf serum, ITS: insulin-transferrin-selenium, FGF: fibroblast growth factor; EGF: epidermal growth factor.

Table S2. Experimental design of the RSM in a randomized order

Run	HSA ($\mu\text{g mL}^{-1}$)	FCS <i>v/v</i> (%)	ITS (mg L^{-1})	FGF-1 (ng mL^{-1})	EGF/FGF-2 (ng mL^{-1})
1	4.0	0.4	1.6	10.0	10.0
2	44.9	0.0	3.9	0.0	10.0
3	74.0	0.0	4.0	6.5	10.0
4	0.0	0.5	2.3	1.0	0.0
5	0.0	0.0	2.0	10.0	10.0
6	48.0	0.0	3.3	10.0	0.0
7	0.0	0.0	4.0	0.0	5.1
8	0.0	0.0	0.0	5.6	0.0
9	80.0	0.5	0.8	10.0	0.0
10	80.0	0.5	0.0	2.2	5.9
11	44.4	0.3	0.0	0.0	0.0
12	47.2	0.5	4.0	0.0	4.9
13	80.0	0.5	4.0	10.0	6.6
14	80.0	0.3	4.0	3.3	0.0
15	0.0	0.4	0.0	0.0	9.8
16	80.0	0.3	4.0	3.3	0.0
17	80.0	0.1	0.0	10.0	5.5
18	40.0	0.3	2.0	5.0	5.0
19	44.4	0.3	0.0	0.0	0.0
20	40.0	0.3	2.0	5.0	5.0
21	80.0	0.3	0.7	5.7	0.0
22	80.0	0.2	1.7	0.0	2.0
23	0.0	0.0	4.0	5.3	10.0
24	40.0	0.5	2.0	5.0	5.0
25	80.0	0.3	2.8	0.0	10.0
26	80.0	0.3	2.8	0.0	10.0
27	40.0	0.3	2.0	5.0	5.0
28	40.0	0.3	2.0	5.0	5.0
29	58.8	0.3	0.1	10.0	10.0
30	0.0	0.5	0.0	10.0	3.5
31	40.0	0.5	2.0	5.0	5.0
32	0.0	0.1	1.9	1.0	10.0
33	0.0	0.3	4.0	10.0	0.0
34	38.3	0.5	1.6	2.7	10.0
35	44.8	0.0	0.0	1.7	10.0
36	80.0	0.0	1.7	0.0	2.0

HLSCs were cultured in M11 on B2 surfaces, with corresponding supplementation for each experimental condition.

RSM: response surface methodology, HLSCs: human liver stem cells, HSA: human serum albumin, FCS: fetal calf serum, ITS: insulin-transferrin-selenium, FGF: fibroblast growth factor; EGF: epidermal growth factor.