Scalable production of size-controlled cholangiocyte and cholangiocarcinoma organoids within liver extracellular matrix-containing microcapsules

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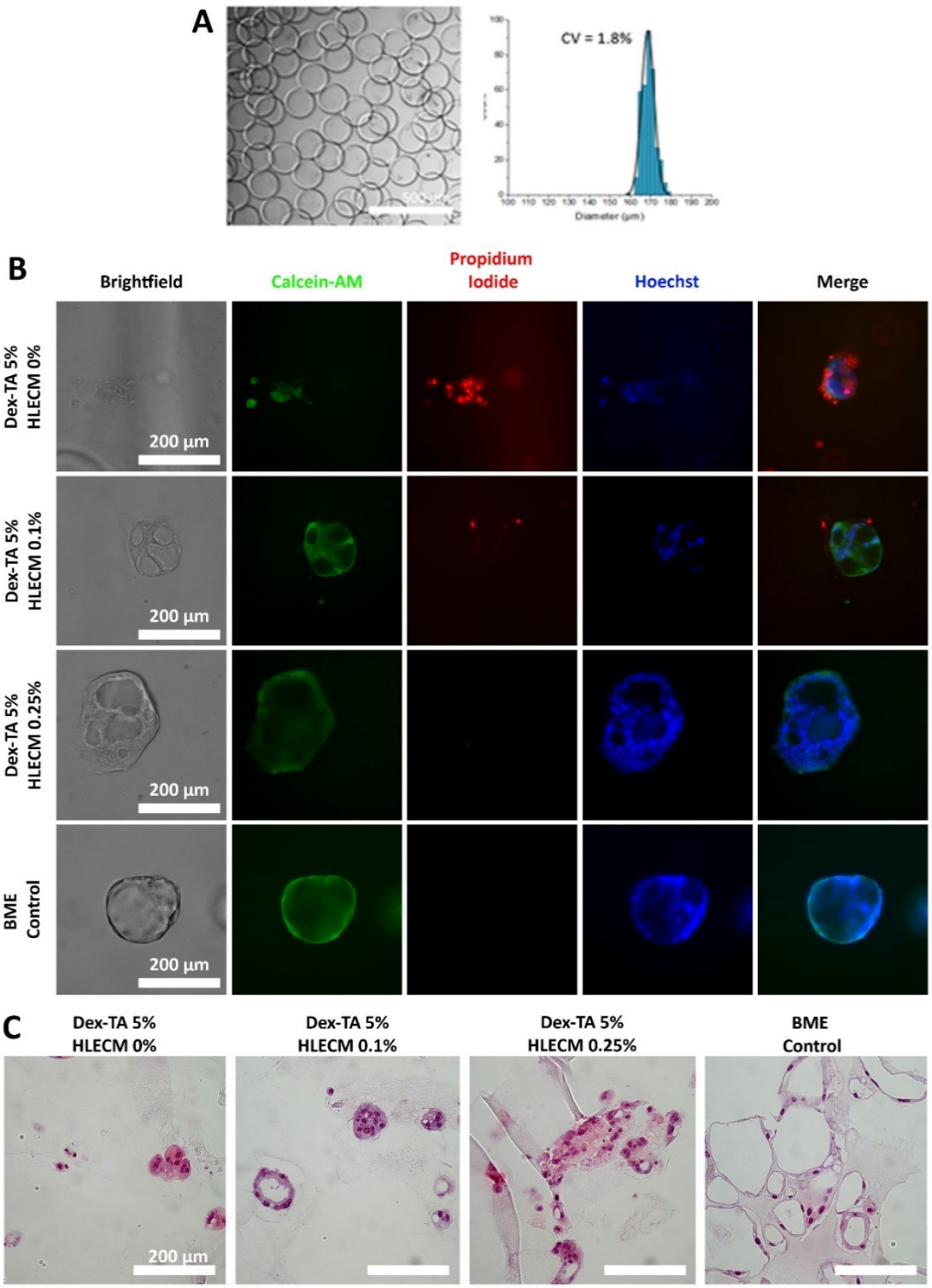
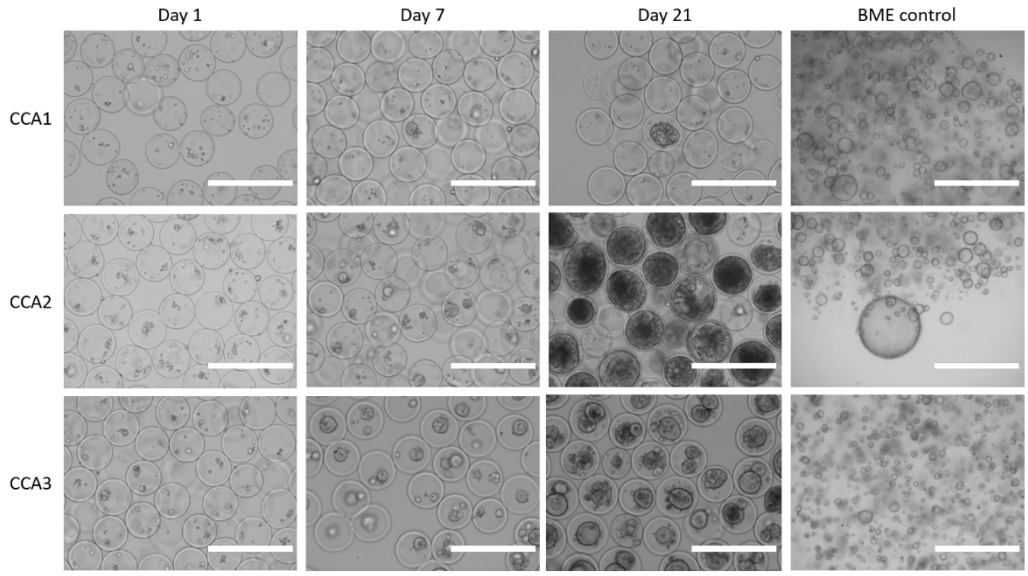


Supplemental Figure S1: ICO can self-assemble within Dex-Ta microgel, but can only survive with the addition of HLECM. **A)** Microcapsules can be produced with high monodispersity (CV=1.8%). **B)** Addition of HLECM to Dex-Ta increases cell viability, **C)** and allows for the self-organization of cells into organoid-like structures.



Supplemental Figure S2. CCAO formation in microcapsules. Representative bright field micrographs of CCAO cultured in BME control for 7 days or cultured in microcapsules for 1, 7, and 21 days. Scale bar indicates 400 µm.

Table S1. Medium components required for the formulation of basal medium (ADV+)

Component	Amount	Concentration	Brand
Advanced DMEM/F12	500ml		Gibco
HEPES	5ml	1M	Life technologies
L-Glutamin	5ml	100X	Life technologies
Primocin	1ml	500mg/ml	Invivogen
Pen/Strep	5ml	10000 U/ml	Life technologies

Table S2. Medium components required for the formulation of Start Up Medium (SEM) or Expansion Medium (EM). Medium components with a * are only added to SEM.

Component	Concentration	Brand
Adv+	-	Gibco
N2	1%	Gibco
B27	2%	Gibco
N-Acetylcystein	1mM	Sigma-Aldrich
gastrin	10 nM	Sigma-Aldrich
EGF	50 ng/ml	Peprotech
FGF10	100 ng/ml	Peprotech
HGF	25 ng/ml	Peprotech
nicotinamide	10nM	Sigma-Aldrich
A83.01	5 µM	Tocris
Forskolin	10 µM	Torcris
R-Spondin	10%	Conditioned medium
WNT*	30% Wnt	Conditioned medium
Noggin*	25 ng/ml	Conditioned medium
Y27632*	10µM	Tocris

hES cell cloning recovery solution*	1:1000 dilution	Stemgent

Table S3. List of primary antibodies used in this study

Primary antibody	Raised in	Dilution	Supplier
ZO1	Rabbit	1:100	Proteintech
KRT7	Mouse	1:100	Dako
KRT19	Mouse	1:100	Dako
KI67	Rabbit	Ready –to-use	Kind gift from pathology

Table S4. List of fluorescent labeled secondary antibodies used in this study

Secondary antibody	Raised in	Against	Dilution	Supplier
Alexa 555	Goat	Mouse	1:100	Fisher scientific
Alexa 488	Goat	Rabbit	1:100	Fisher scientific

Table S5. List of qPCR primers used in this study

Primer	Species	Forward sequence 5'to 3'	Reverse sequence 5'to 3'
GAPDH	Human	CTTTTGCGTCGCCAGCCGAG	CCAGGCGCCCAATACGACCA
HPRT1	Human	ACCAGTCAACAGGGGACATAA	CTTCGTGGGGTCCTTTTCACC
B2M	Human	GTGTCTGGGTTTCATCCATC	GGCAGGCATACTCATCTTTT
LGR5	Human	GTCAGCTGCTCCCGAATCCC	TGAAACAGCTTGGGGGCACA
PROM1	Human	CCTGGGGCTGCTGTTTATTA	ATCACCAACAGGGAGATTGC
KRT7	Human	GGGGACGACCTCCGGAATAC	CTTGGCACGCTGGTTCTTGA
KRT19	Human	GCACTACAGCCACTACTACACGA	CTCATGCGCAGAGCCTGTT
EPCAM	Human	GACTTTTGCCGCAGCTCAGGA	AGCAGTTTACGGCCAGCTTGT
MUC1	Human	CTGTCACTGCCGCCGAAGA	CGTGCCCCTACAAGTTGGCA
KI67	Human	CTACGGATTATACCTGGCCTTCC	AGGAAGCTGGATACGGATGTCA
Vimentin	Human	CGGGAGAAATTGCAGGAGG	TGCTGTTCCTGAATCTGAGC
Albumin	Human	CTGCCTGCCTGTTGCCAAAGC	GGCAAGGTCCGCCCTGTCATC
CYP3a4	Human	AGCAAAGAGCAACACAGAGCTGAA	CAGAGGTGTGGGCCCTGGAAT
HNF4α	Human	GTACTCCTGCAGATTTAGCC	CTGTCCTCATAGCTTGACCT
ITGB1	Human	GGACGCCGCGCGGAAAAGAT	CACCCACAATTTGGCCCTGCT
ITGA5	Human	AGGGTCGGGGGCTTCAACTTA	GAGCGGCAGGGTGCATACTC