Electronic Supplementary Material (ESI) for Lab on a Chip.
This journal is © The Royal Society of Chemistry 2023

1 **Acrylic-based Culture Plate Format Perfusion Device**

2 **to Establish Liver Endothelial-Epithelial Interface**

3

4 Dennis McDuffie[1], Bhumi Suthar[1], Charles G. Alver[1], Madeline Helm[1], David Oliver[1],

5 R. Alan Burgess[2], David Barr[2], Emmanuel Thomas[2,3*] and Ashutosh Agarwal[1,3,4*]

6

7 [1]Department of Biomedical Engineering, University of Miami, Coral Gables, FL, USA

8 [2]Department of Pathology & Laboratory Medicine, Schiff Center for Liver Diseases, University

9 of Miami Miller School of Medicine, Miami, FL, USA

10 [3]Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine

11 [4]Desai Sethi Urology Institute, University of Miami Miller School of Medicine

12

13

14

15 **Corresponding Authors:**

16 Emmanuel Thomas, M.D., Ph.D., FAASLD

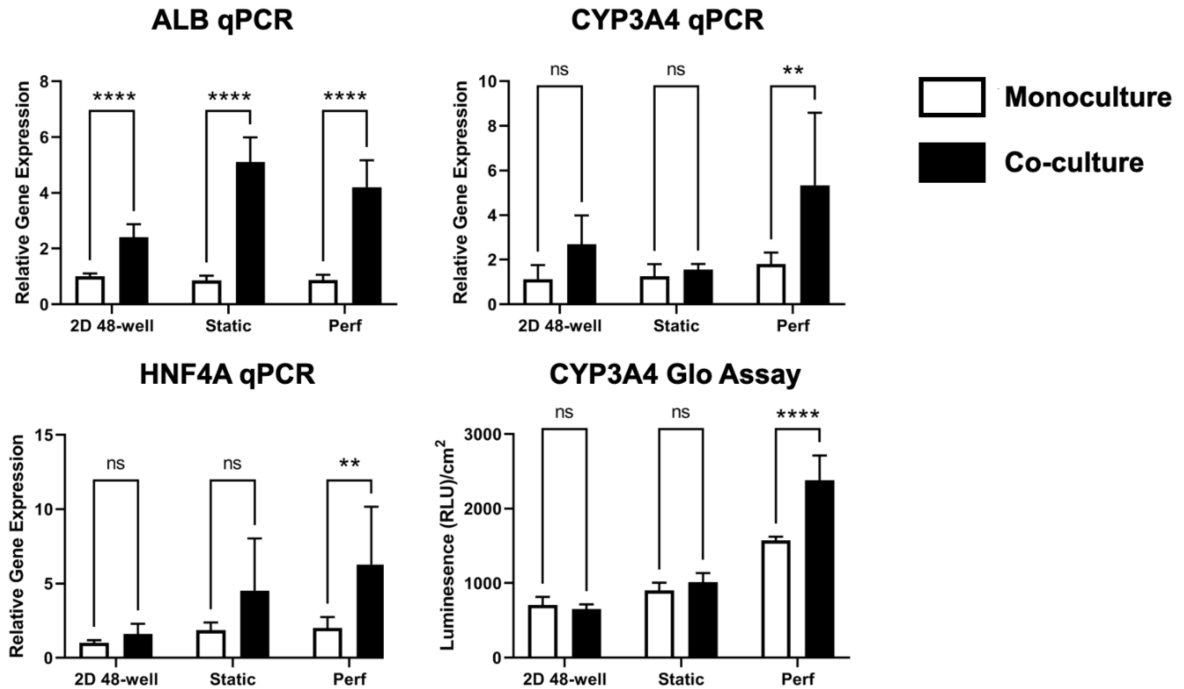
17 TEL: +1 305 243-2895

18 E-mail: Ethomas1@med.miami.edu and

19 Ashutosh Agarwal, Ph.D.

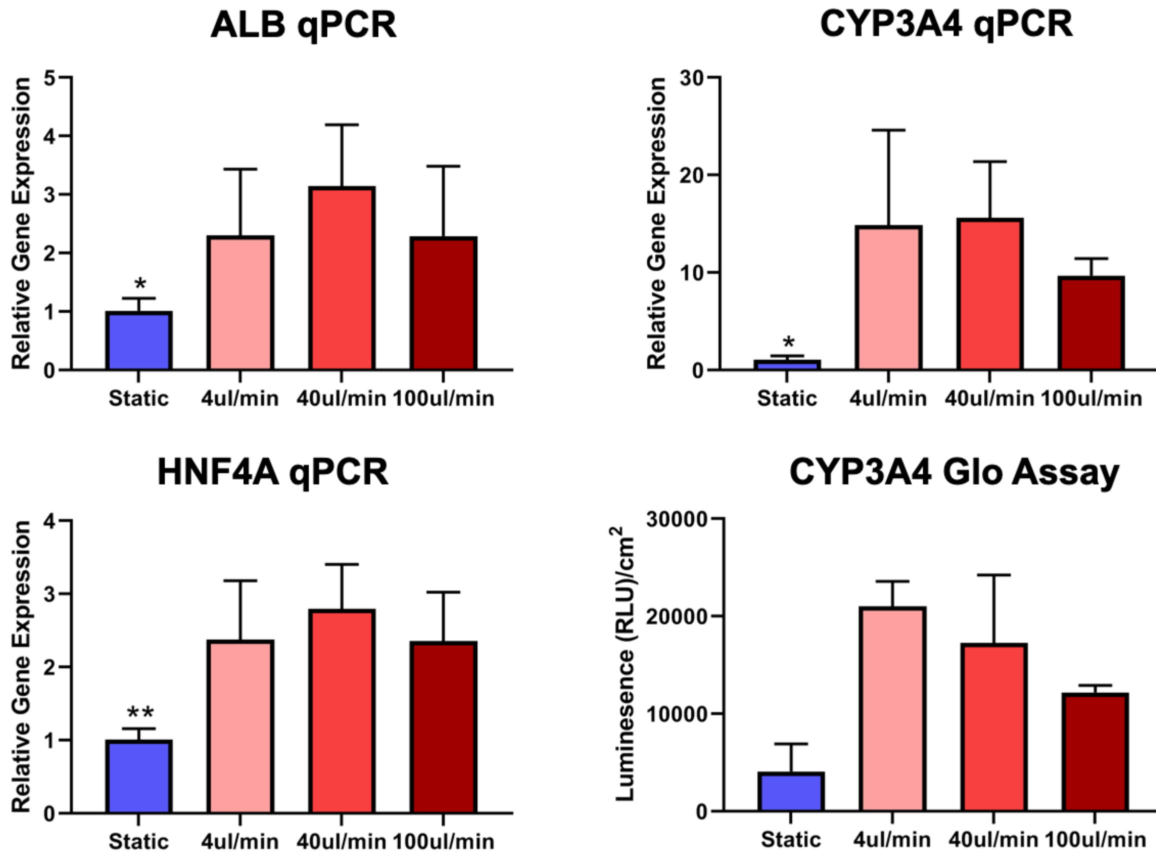
20 TEL: +1 305 243-8925

21 E-mail: A.agarwal2@miami.edu



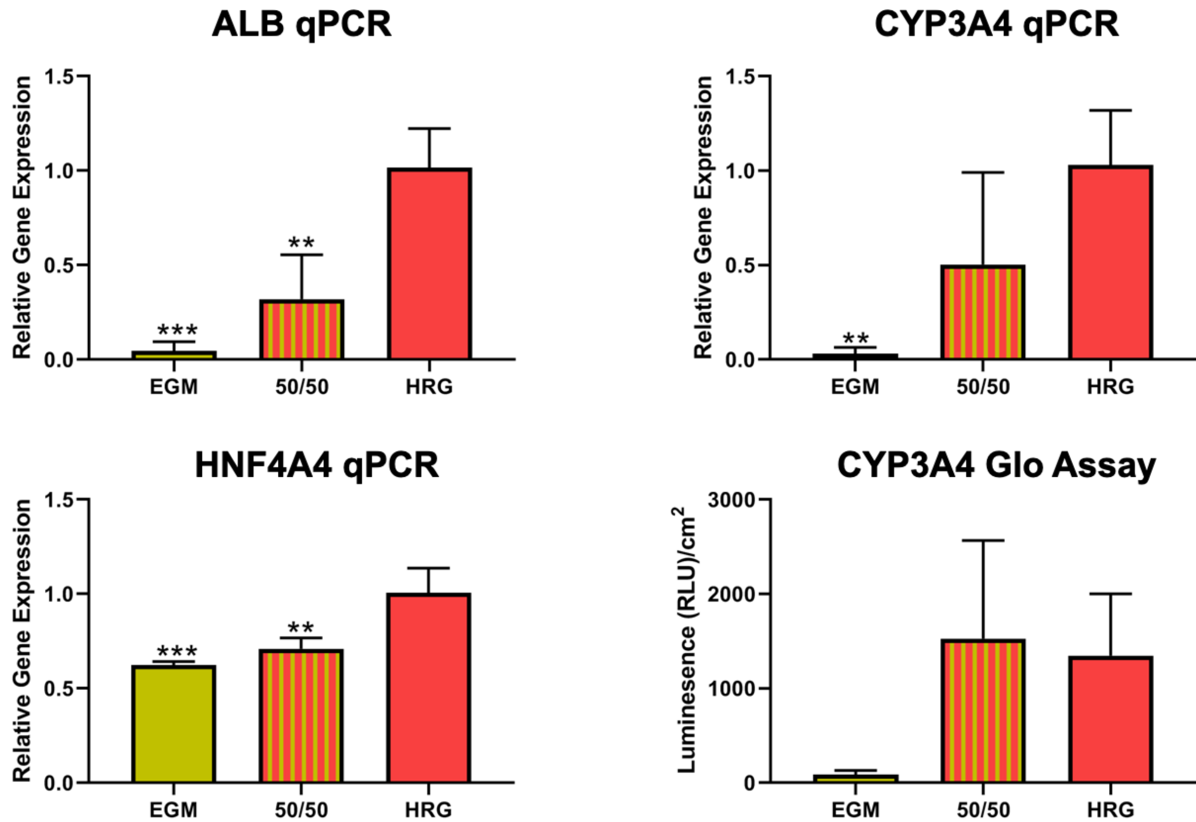
22

23 **Supplementary Figure 1: Monoculture vs. Co-Culture.** Each culture platform (2D, static MPS,
24 and perfusion MPS) was compared while seeded with monocultures and co-cultures for 7 days and
25 assessed on hepatocyte differentiation marker expression. The co-culture conditions were seeded
26 with HUVECs 1 day prior to HepaRG seeding. The monoculture MPS conditions did not have any
27 cells on the underside of the membrane, only HepaRGs on the top side of the membrane. The
28 perfusion MPS co-culture condition demonstrated significantly higher expression of all
29 differentiation markers of interest. HUVEC-HepaRG co-cultures were used in the static and
30 perfusion MPS conditions for all other experiments. Statistical significance was determined using
31 two-way ANOVA and Bonferroni's post-test ($n = 4$) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ****
32 $p < 0.0001$.



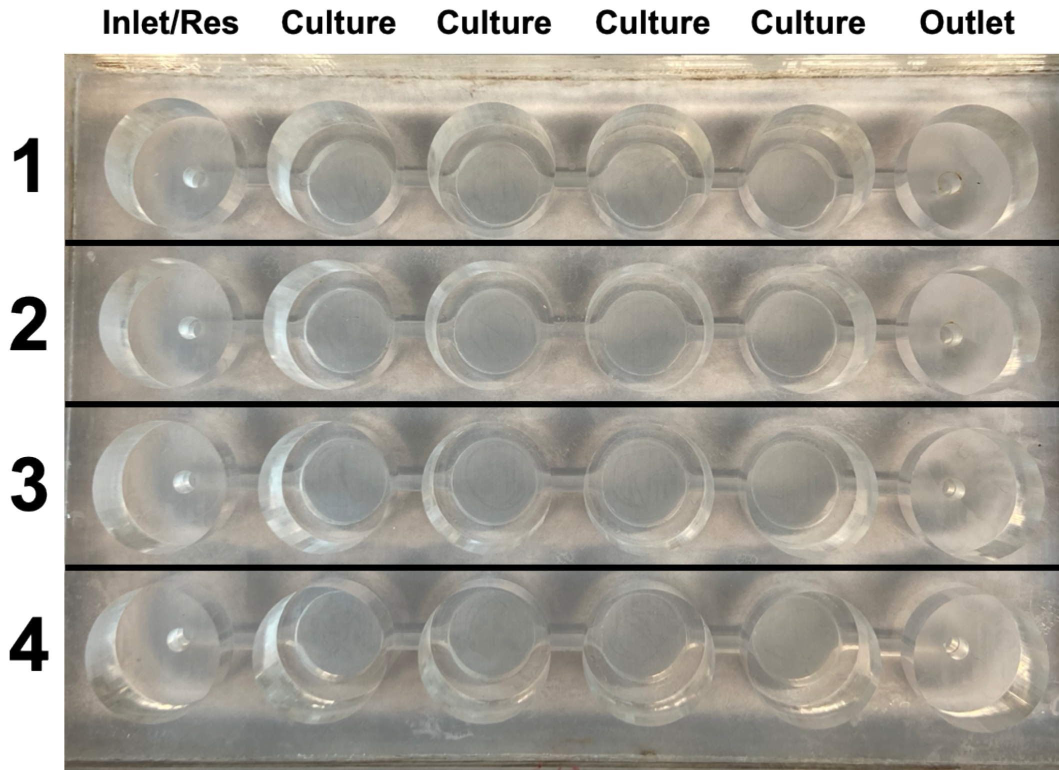
33

34 **Supplementary Figure 2: Flow Rate Comparison.** 3 different perfusion flow rates (4µL/min, 35 40µL/min, 100 µL/min) for the MPS co-culture condition were compared for 7 days and assessed 36 on hepatocyte differentiation marker expression. The presence of perfusion had a greater impact 37 on marker expression than changing the flow rate did, but across all markers, the 40µL/min flow 38 rate showed the highest degree of differentiation. 40µL/min was selected as the perfusion rate for 39 all other experiments. Statistical significance was determined using one-way ANOVA and 40 Tukey's *post hoc* test. Significance is given with respect to the 40µL/min condition (n = 4) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

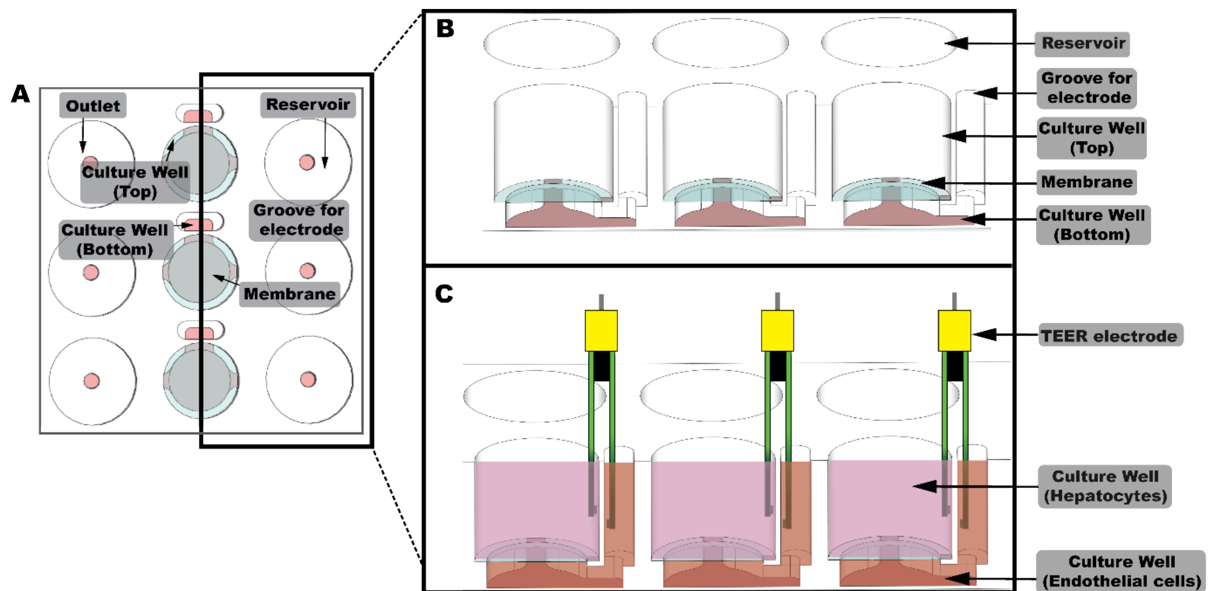


42
43 **Supplementary Figure 3: Media Comparison.** 3 different media combinations (all endothelial
44 media, 50/50 combination, all HRG media) for the perfusion MPS co-culture condition were
45 compared for 7 days and assessed on hepatocyte differentiation marker expression. The all HRG
46 media elicited significantly higher expression of albumin and HNF4A compared to both other
47 media combinations, and was not significantly different than the 50/50 media in terms of
48 CYP3A4 expression and activity. Only HRG media was used in all other experiments after
49 HepaRG seeding was complete. 2D co-cultures also only used HRG media. Statistical
50 significance was determined using one-way ANOVA and Tukey's *post hoc* test. Significance is
51 given with respect to the all HRG condition (n = 4) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ****
52 $p < 0.0001$.

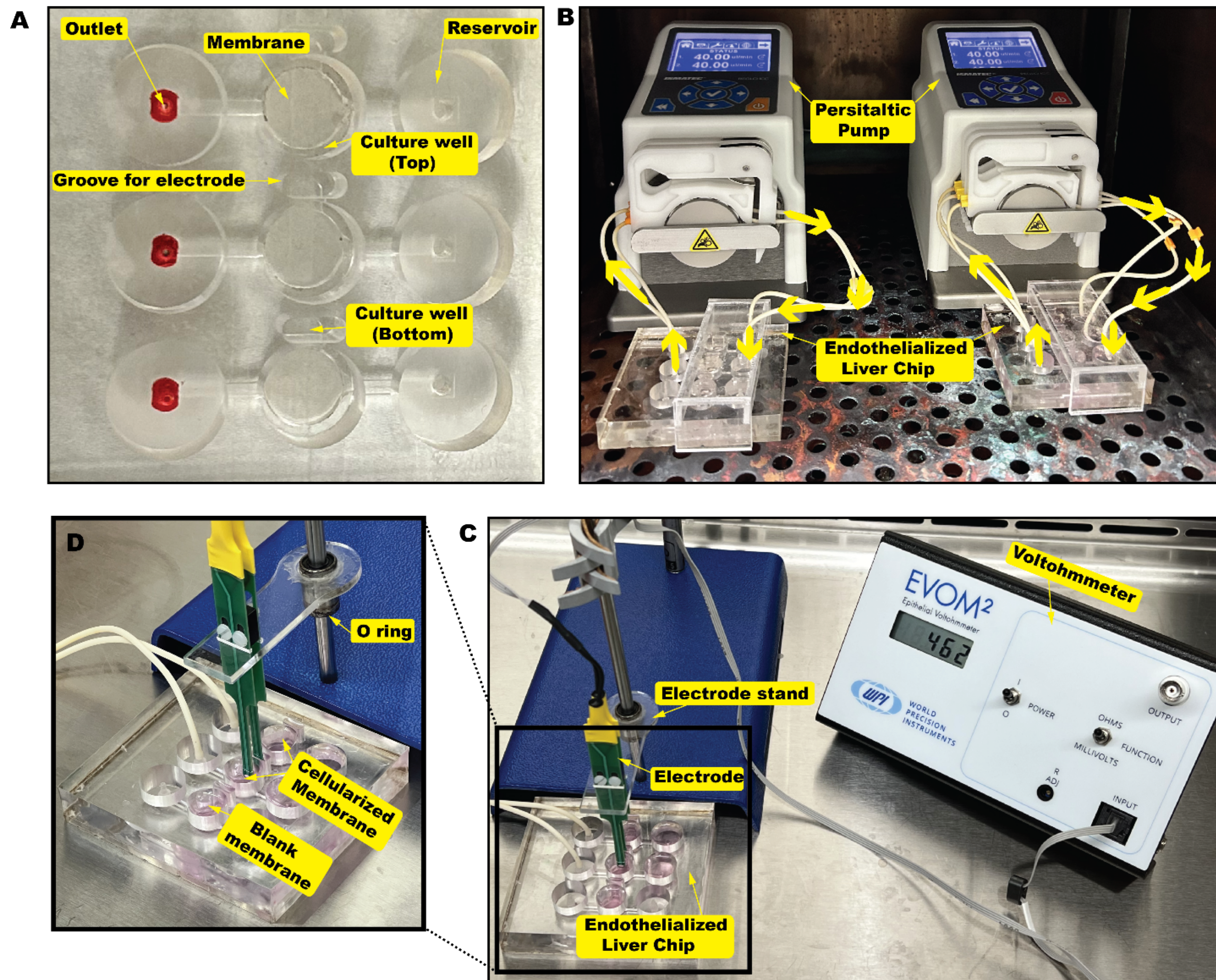
53



54 **Supplementary Figure: 4 Multi-Organ MPS.** This concept design links 4 separate culture
55 wells on which 4 different tissues could theoretically be cultured in quadruplicate. This assembly
56 could be used to test downstream effects of treatments on multiple different organs. The inlet
57 well could serve as both an inlet (if media is not recirculating), or as a reservoir (if media is
58 recirculating). The generic MPS can be altered and made more complex to accommodate
59 different applications.
60



61
62 **Supplementary Figure: 5 Chip modifications for TEER measurements in situ.** The original
63 design was tweaked to enable in situ TEER measurements using chopstick electrode. The design
64 parameters such as the dimensions of the culture, reservoir, & outlet well remain the same. (A)
65 The prototype shown here features 3 outlet wells (left), 3 culture wells (middle), 3 grooves for
66 electrode, & 3 reservoir wells (right). (B) The inset shows a cross sectional view of the modified
67 chip. The top piece incorporates a groove to allow introduction of the TEER electrode. The culture
68 well of the bottom piece is slightly extended towards the groove allowing the electrode to access
69 the culture media. (C) The schematic shows the placement of TEER electrodes in top and bottom
70 culture well.



71
72 **Supplementary Figure 6: Chip and TEER measurement setup.** (A) Acrylic based chip was
73 milled featuring- outlet, reservoir wells, culture wells and a groove for electrode as shown. (B)
74 The chip setup consists of a peristaltic pump connected to the chip via peristaltic tubing. An acrylic
75 well casing to protect culture and reservoir wells whilst securing the tubing placement. The arrows
76 show the direction of the media flow. (C) TEER setup comprises of STX3 chopstick electrodes
77 connected to an EVOM^2 volt/ohm meter (World Precision Instruments). An electrode stand was
78 fabricated with acrylic to ensure that electrodes are perpendicular to the membrane thus avoiding
79 angle variance. (D) The depth of the electrodes immersed in culture media can be kept constant
80 for each reading by employing an O ring below the stand to avoid depth variance. Resistance of
81 the cell layer (R_{cells}) is obtained by deducting the measured resistance (R_{meas}) (membrane with
82 cells) from the resistance of the blank (R_{blank}) (membrane without cells) i.e. $R_{cells} = R_{meas} - R_{blank}$.
83 To calculate the TEER value, we multiply the R_{cells} value with the area of membrane (1.727 cm^2)
84 i.e. $TEER = R_{cells} \times M_{area}$.