A perfusion incubator liver chip for 3D cell culture with application on chronic hepatotoxicity testing

Supplementary Information

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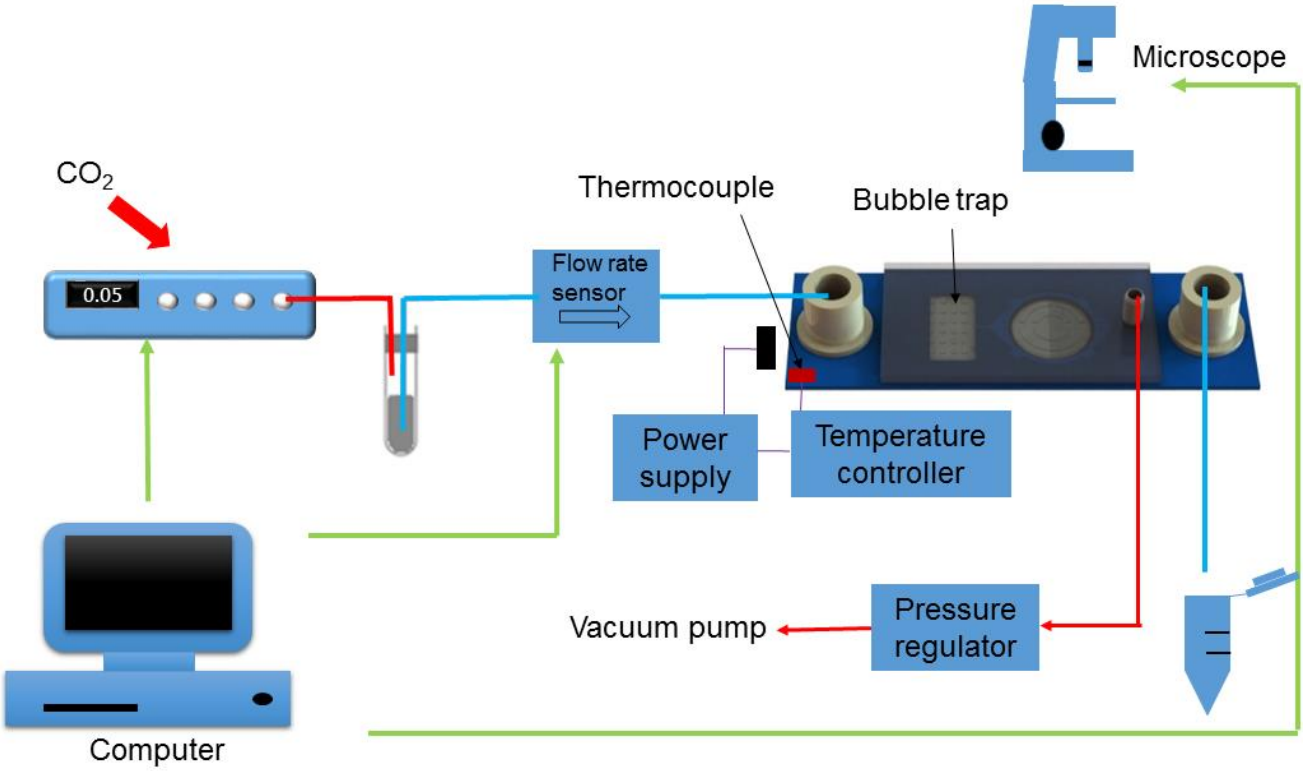


Figure S1: overall schematics of the microfluidic system

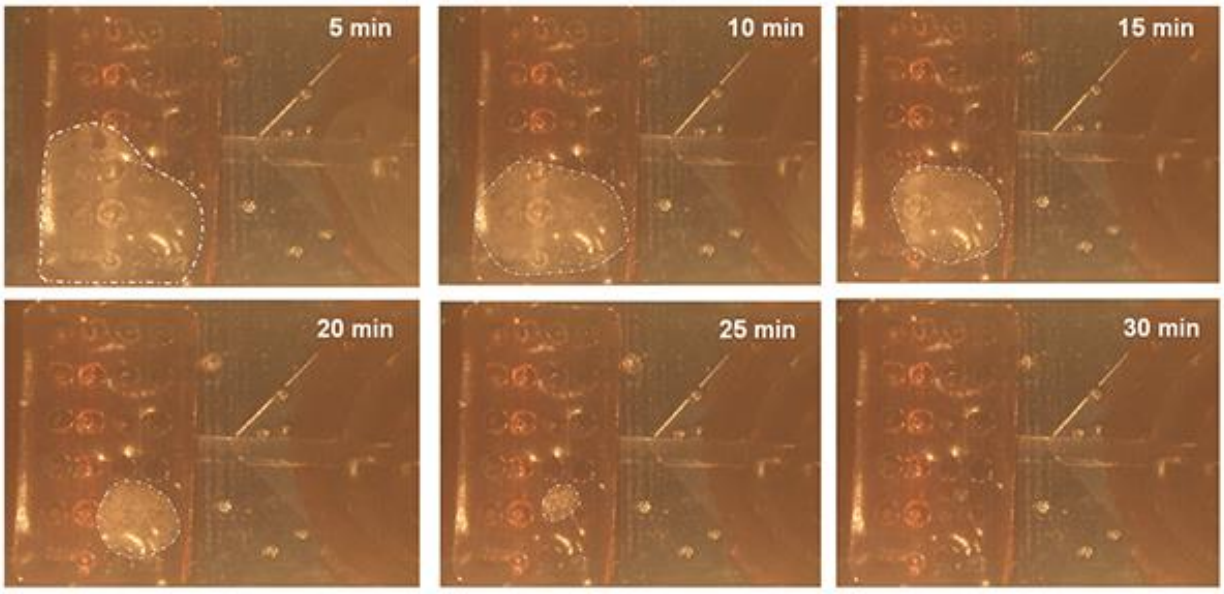


Figure S2: removal of the air bubble (shown in the dotted line) in the bubble trap. The bubble is completely removed after 30 minutes.

Supplementary 3: Influence of the cell culture chamber's depth:

Different depth of the cell culture chamber: 0.5mm, 1mm, 2mm and 3mm were fabricated in order to appreciate the relevance of this parameter The devices having 0.5 and 1mm chamber depth were fabricated as described in previous section. The chips having 2mm and 3mm chamber depth were fabricated assembling one or two silicon "spacers" -1mm-thick (with the corresponding etch-through holes). The assembly was performed using a thin PDMS layer using a contact imprinting method [73]. The experiments were performed for 3 days at a constant flow rate of 0.1mL/h. Cell viability (Fig. S3b) is not affected, but for albumin secretion (Fig. S3c) and urea synthesis (Fig. S3d), the cells cultured in chamber with 0.5mm depth showed the highest secretion level. Fig. 4a with Fig. 5a and Fig. S3b, show that the O_2 level increases sharply from Q=0.02mL/h to Q=0.06mL/h, and then increases moderately to Q=0.1mL/h. Correspondingly, cell viability increases sharply from Q= 0.02mL/h to Q = 0.06mL/h; and then moderately to Q =0.1mL/h before it decreases at Q=0.2 and 0.4mL/h due to excessive shear stress. On the other hand, the change of depth from 0.5mm to 3mm less significant effect on the O_2 level.

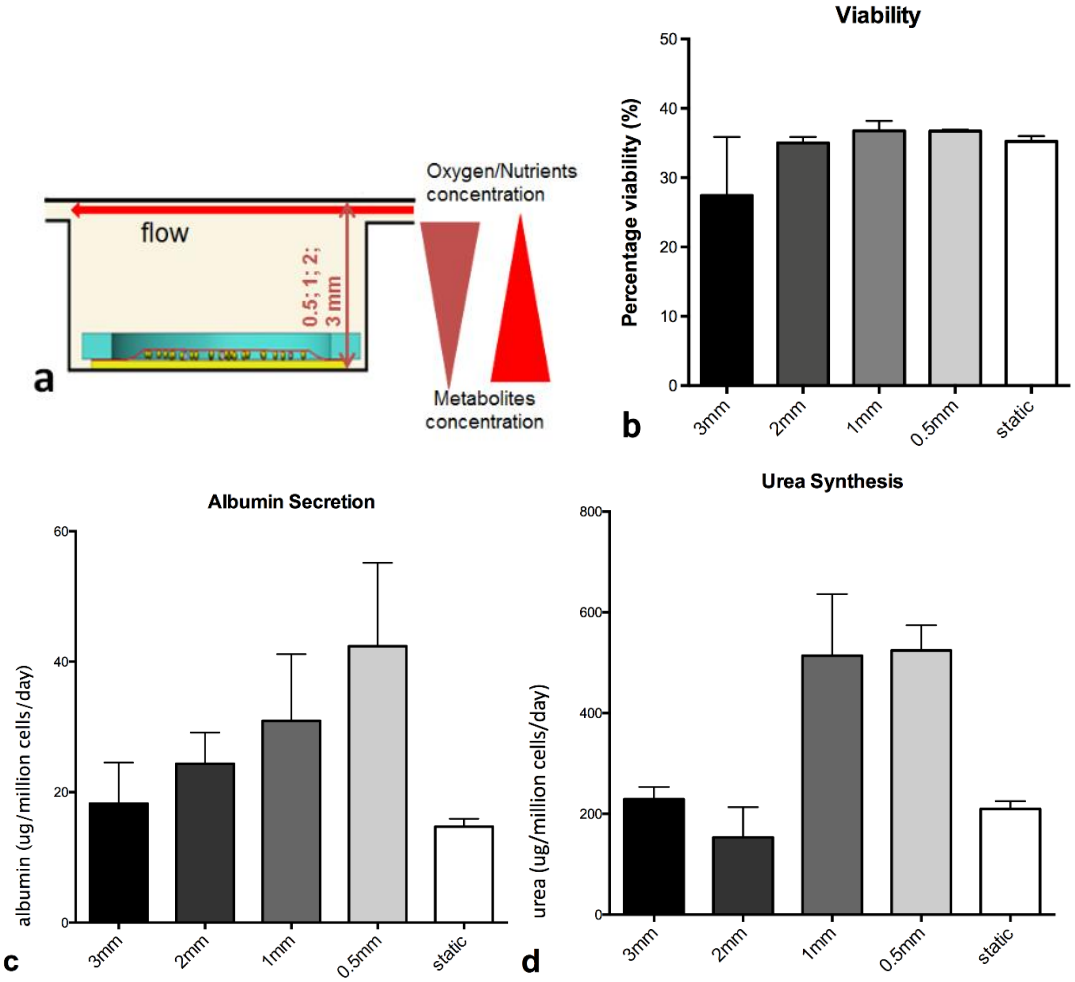


Figure S3: a) Schematic design with the positioning of the CS model in the microwell; the concentration of the O_2 and nutrients decrease with the depth of the chamber culture. S3b) Cell viability was not significantly different for different chamber depths, cell number was normalized to the initial cell number. S3c) Albumin production was highest at 0.5mm d) urea synthesis was highest at 1 mm and 0.5 mm. Results were obtained from triplicate measurements (n=3), *p≤0.05. Data from representative experiments are presented, whereas similar trends were seen in multiple trials.

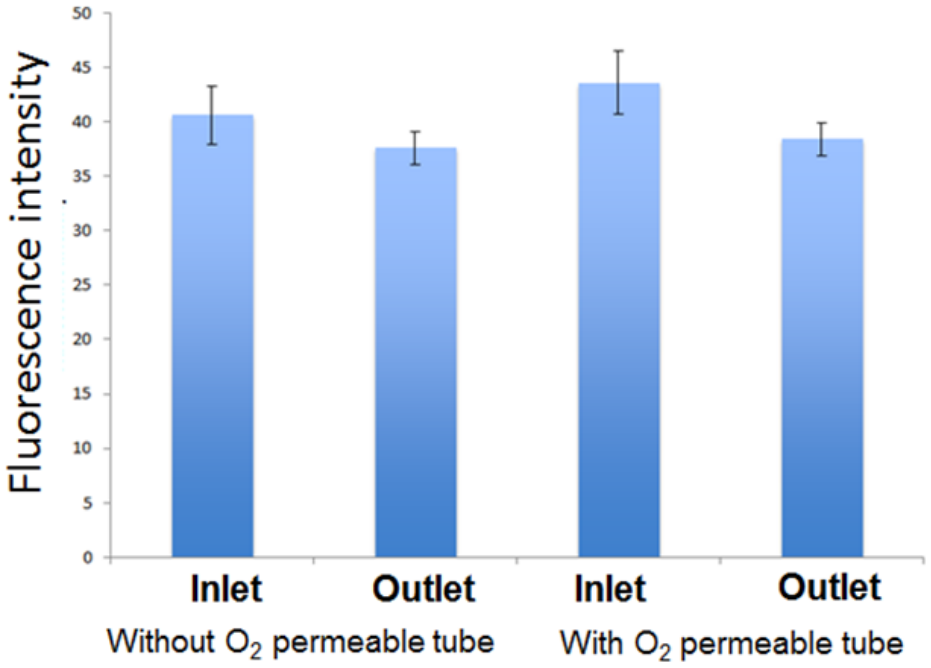
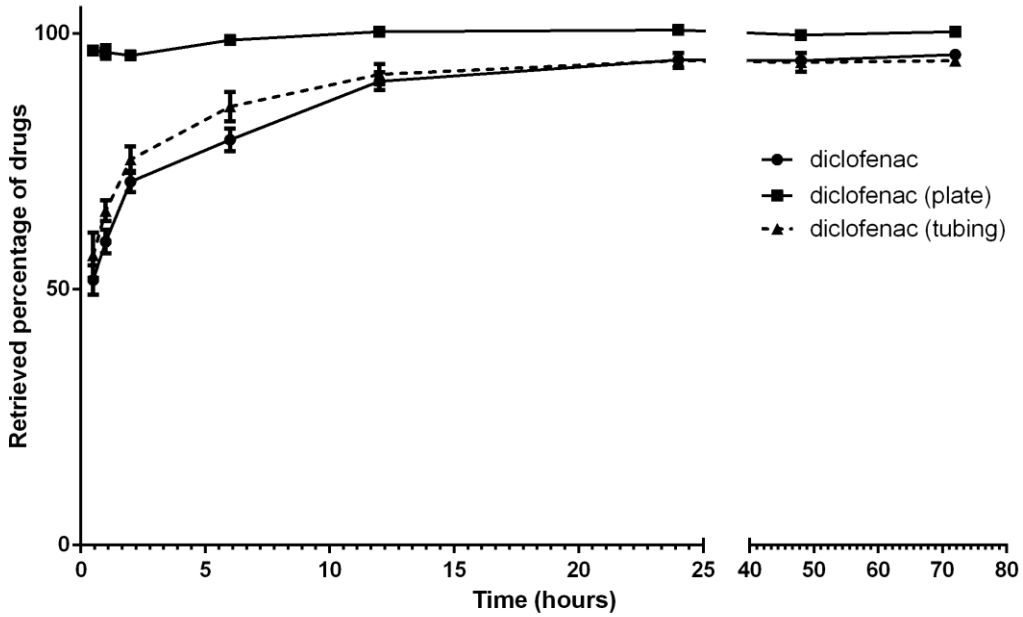


Figure S4: The drop in oxygen concentration in the media after flowing at 0.1mL/h through the PIC system loaded with rat hepatocytes spheroids (~2 x 10^4 cells) in the presence/absence of oxygen permeable tube

a



b

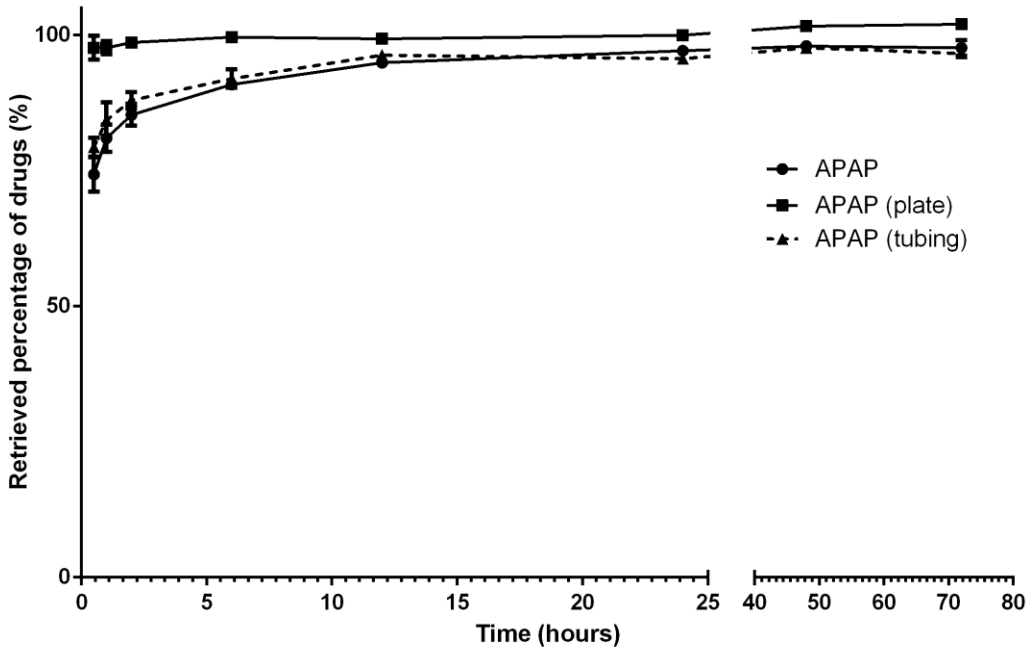


Figure S5: Amount of (a) diclofenac and (b) APAP retrieved after flowing through the PIC, tubing system (without PIC) and well-plate, normalized to the total amount of drug loaded into PIC, tubing and well-plate for 0.5 to 72 hours. Results are the average ± the standard error of the mean collected from 3 different experiments.