Supplementary Information for:

Hepatic Organoids for Microfluidic Drug Screening

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A critical aspect of the general automated droplet exchange procedure (GODEP) described in the main text is mixing. To examine this process, a dye mixing experiment was devised (Fig. S1A). 630 nL PBS droplets containing blue food dye (representing media containing organoids) were positioned adjacent to retention barriers. 1.36 µL droplets of PBS were then dispensed and merged with the dye-containing droplets by GODEP. Linear actuation of the merged droplet from the nearest 2.2 x 2.2 mm electrodes to the small reservoirs and back again constituted one mix cycle. A total of 6 mix cycles was conducted with photos collected immediately after mixing and at the end of each mix cycle (as depicted in Fig. S1A) for analysis with ImageJ software. Four regions of interest (ROI) were defined encompassing the majority of each of four merged droplets. The images were split into red, green and blue channels, and the histogram function was used to evaluate the standard deviation of dye intensity in the ROI in the red channel as an estimate of unmixed heterogeneity. The unbiased estimate of the standard deviation was used to estimate the standard deviation of the sample deviation.[1] As shown in Figure S1B, these data demonstrate that merged droplets are well mixed within 2 cycles (4 total paths across 5 linear electrodes). Nonetheless, a total of 5 cycles (10 paths) was chosen for all experiments described here to ensure complete mixing. At the speeds used here, 5 cycles of mixing was typically complete in ~30 s.

The results in Figure S1B suggests that the process of droplet movement causes convective/advective mixing, which has been described previously.[2] In addition, the retention barriers may have contributed to the mixing efficiency by introducing hydrodynamic instabilities.[3]
References


**Figure S1.** Dye-mixing study to characterize the mixing efficiency of the general organoid droplet exchange procedure (GODEP). (A) Sample frames from a video depicting the mixing experiment. 1) Feed droplets dispensed and aligned with dye-containing droplets. 2) Feed and dye droplets merged. 3) First mix cycle begun by actuating merged droplets towards small reservoirs. 4) First mix cycle ended by actuating merged droplets onto 2.2 x 2.2 mm electrode. 5) Second mix cycle begun. 6) Second mix cycle ended. (B) Standard deviation of red channel intensity within merged droplets at the end of each mix cycle. Error bars represent the unbiased estimate of the standard deviation of the sample standard deviation (n=4).