

Validation of minimally-drug-absorbing thermoplastic Chip-R1 Organ-Chip consumable for assessment of liver metabolism and predictive toxicology

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Abstract #579

Objective

Drug development is a lengthy and expensive process, often taking over 10 years and costing ~\$2B to bring a new drug to market (Deloitte, 2023). Current preclinical safety testing relies on conventional in vitro models that often fail to adequately replicate the in vivo 3D architecture and physiological environment or animal models that do not accurately predict human responses. These limitations result in poor clinical translation, with around 90% of drugs failing in clinical trials (Mullard, 2016).

To create a more human-relevant research model, Emulate has previously developed the Chip-S1® Stretchable Chip and associated organ models, including the liver, kidney, duodenum, and colon. The Chip-S1 is made from polydimethylsiloxane (PDMS), a material chosen for its biocompatibility, gas permeability, optical clarity, and stretchability, enabling researchers to model the mechanical forces induced by breathing or peristalsis. However, PDMS has the potential for drug absorption, which can diminish the system's ability to accurately predict the toxicity, efficacy, and pharmacokinetics for a subset of hydrophobic small-molecule drug candidates.

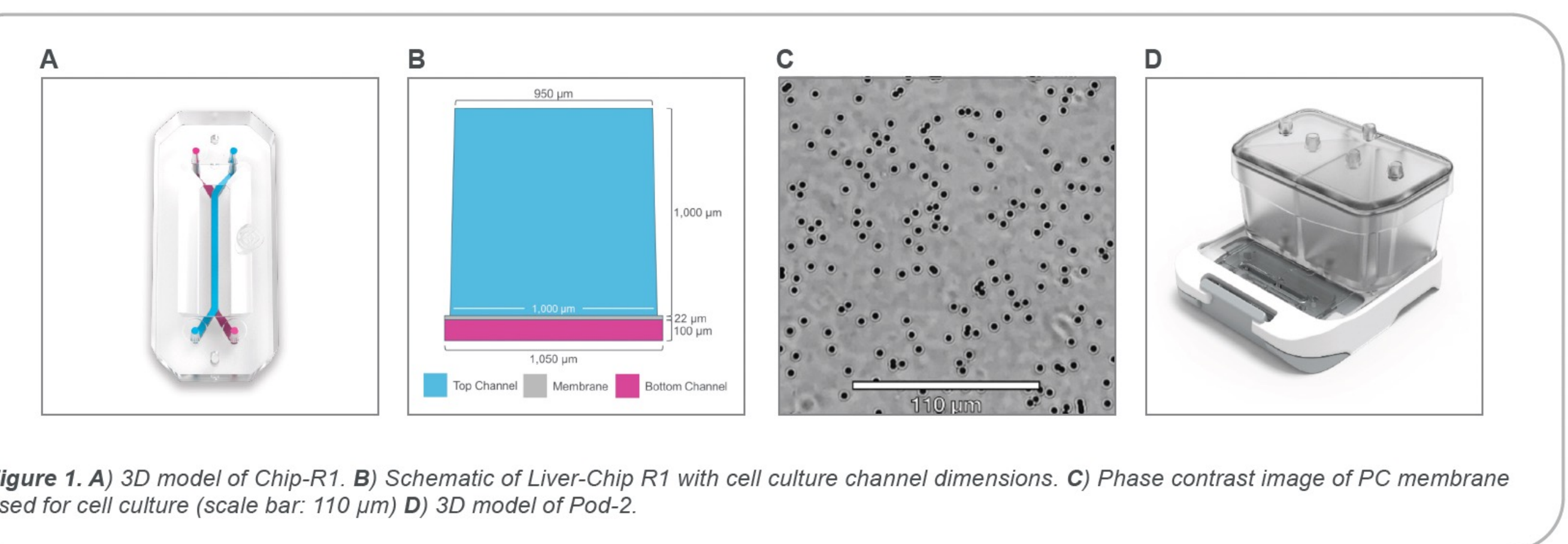
To address these challenges, Emulate developed the Chip-R1 Rigid Chip, which minimizes drug absorption while maintaining the essential architecture of Chip-S1. This enables researchers to build biologically complex Organ-Chip models for tissues that do not require stretch (e.g., liver, kidney, brain, and lung airway). Here, we describe the development of the Chip-R1 Rigid Chip and the Liver-Chip R1 organ model, including data from an equivalency study that demonstrates its utility in modeling the liver and predicting drug hepatotoxicity.

Chip-R1 and Pod-2 Design

The Chip-R1 maintains the two-channel architecture of the Chip-S1 and has several enhancements to improve performance, including updated chip materials, decreased vascular channel height, and reduced chip membrane pore diameter. The system also includes an updated media cartridge, the Pod-2 Portable Module.

Key updates include:

- **Minimal drug absorption**
- **Increased maximum shear stress:**
- **Updated membrane:**
- **Simplified workflow:**



Liver-Chip R1 Model Characterization

An equivalency study was performed to assess the biological performance of the Chip-R1 compared to the Chip-S1, using the Liver-Chip as the reference biological model.

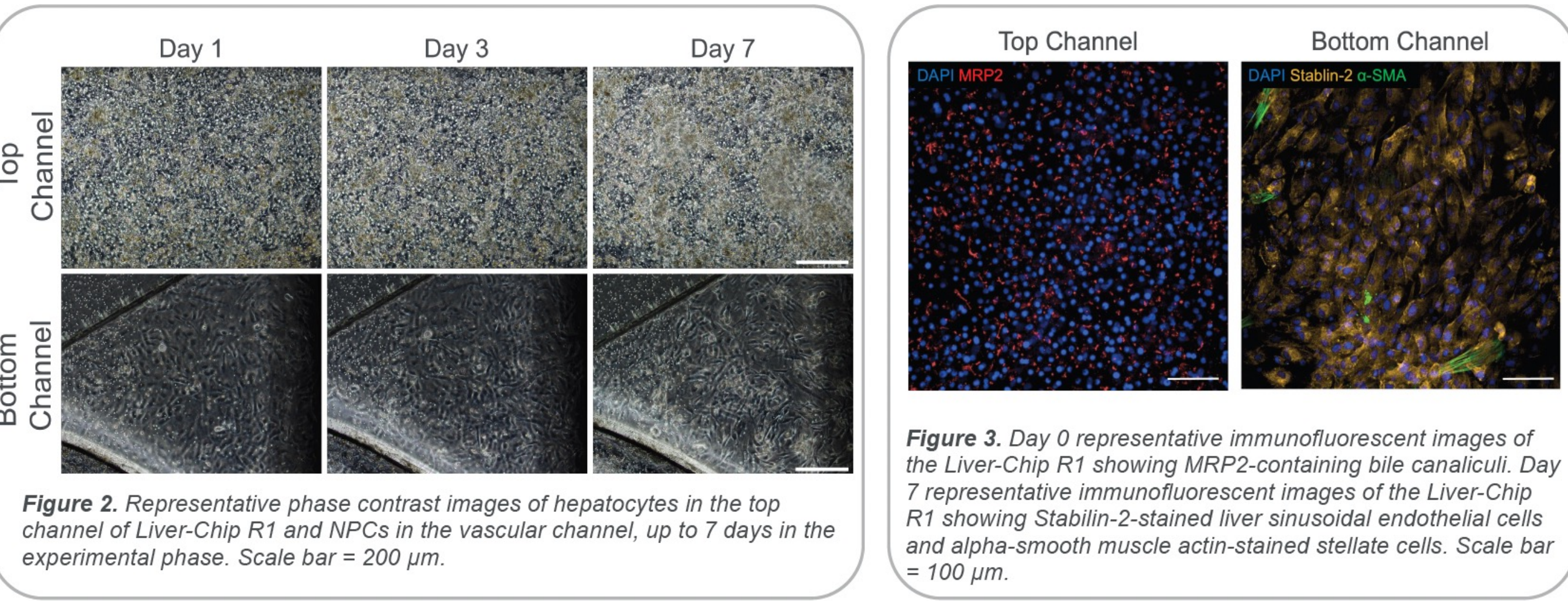
To prepare the Liver-Chip R1, the top and bottom channels were coated with a tissue-specific extracellular matrix (ECM) before primary human hepatocytes were seeded in the top channel. On the same day as hepatocyte seeding, a Matrigel overlay was introduced in the top channel to promote a three-dimensional matrix for the hepatocytes to grow in an ECM sandwich culture.

After an incubation period of 1 day, the bottom channel was seeded with non-parenchymal cells (NPCs)—specifically primary human liver sinusoidal endothelial cells (LSECs), Kupffer cells, and stellate cells. This arrangement emulates the in vivo hepatocyte-sinusoidal endothelial interface. After incubating for 4 hours, chips were then placed in Zoë Culture Module under 30 µL/h media flow for the duration of the experiment.

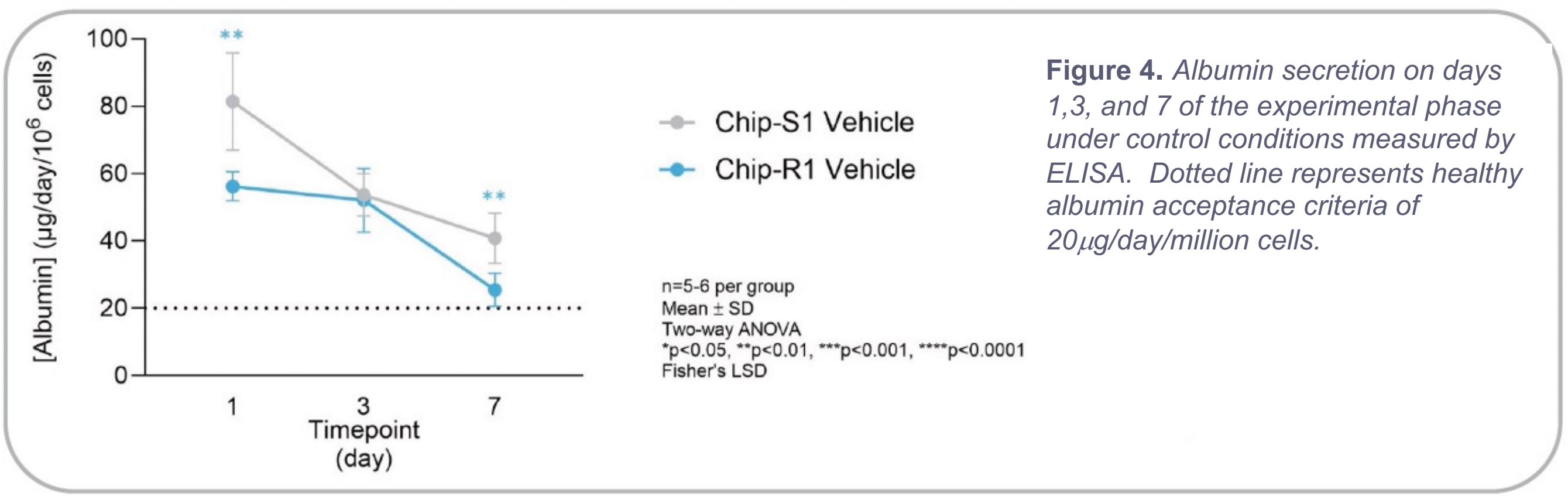
After the 5-6 day stabilization period, the Liver-Chip models were assessed for liver-specific functionality over 7 days (monitoring phase). All readouts are referenced to timepoint days (TPD), with TPD0 being the beginning of this experimental phase. Analysis showed that both the Liver-Chip R1 and Liver-Chip S1 replicated key structural and functional aspects of the liver sinusoid for up to 7 days of monitoring, as assessed by morphology, immunofluorescent staining, albumin production, and liver metabolic activity.

Phase contrast microscopy of Liver-Chip R1 revealed a healthy monolayer of hepatocytes, indicated by a cuboidal and binucleated morphology, and non-parenchymal cells, evident by a confluent monolayer in the bottom channel (see Figure 2).

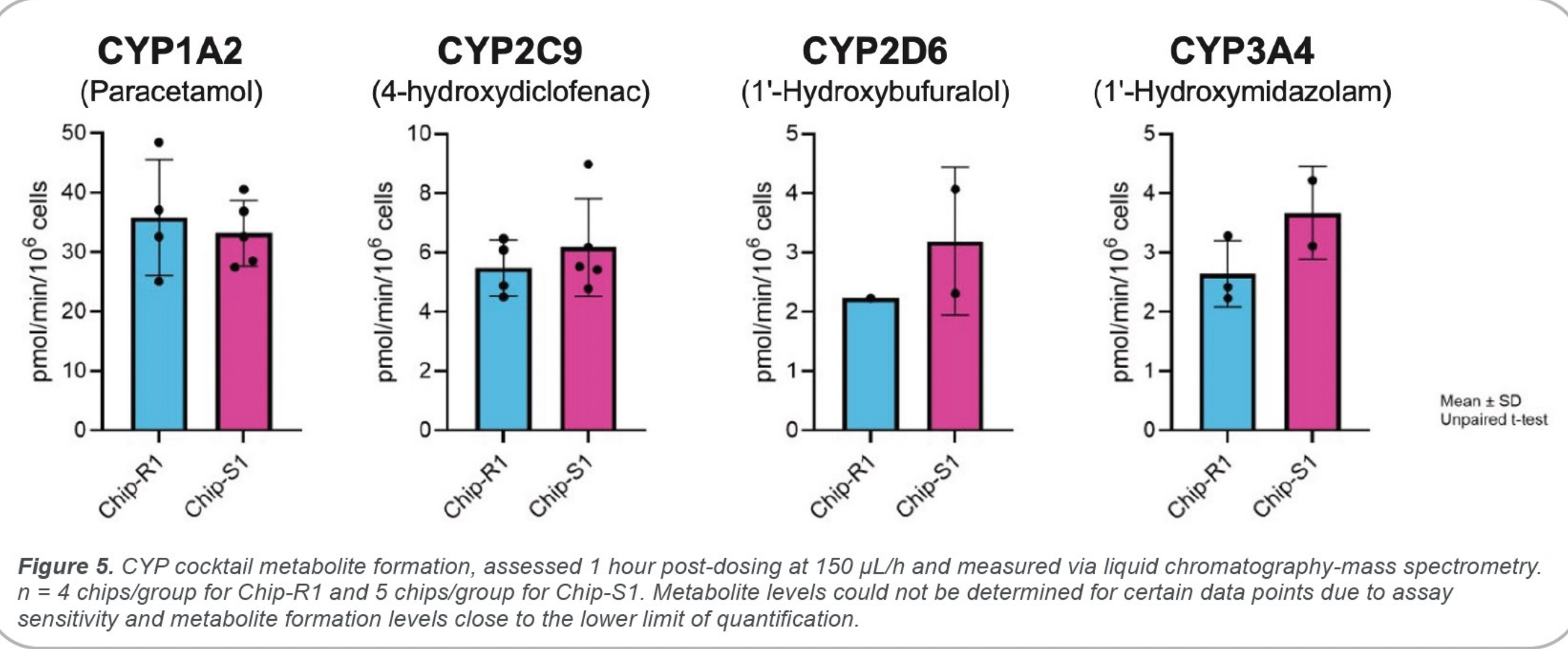
Confocal immunofluorescence microscopy confirmed liver-specific morphological structures, indicated by the presence of multidrug resistance-associated protein 2 (MRP2), Stabilin-2 expression in LSECs, and alpha-smooth muscle actin expression in stellate cells (see Figure 3).



The baseline fidelity and robustness of Liver-Chip R1 was assessed compared to Liver-Chip S1, utilizing albumin production as a key functional biomarker of hepatocellular health. The Liver-Chip R1 demonstrated physiologically relevant levels of albumin synthesis (threshold minimum of 20 µg per day per million hepatocytes) for up to 7 days during the experimental phase under control conditions (see Figure 4).



Functionality of the Liver-Chip R1 was further assessed by measuring the activity of key cytochrome P450 (CYP) metabolizing enzymes (see Figure 5). A cocktail preparation of four probe substrates (phenacetin, diclofenac, bufuralol, and midazolam) was administered to determine metabolic competency of the major CYP isoforms (CYP1A2, CYP2C9, CYP2D6, and CYP3A4, respectively) associated with drug metabolism. This drug cocktail was flowed on chip at 150 µL/h for 1 hour to minimize the potential for metabolite-induced feedback inhibition from the metabolizing enzymatic activity. LC/ MS assessment of parent-to-metabolite production showed comparable levels of enzymatic activity between Liver-Chip R1 and Liver-Chip S1. These results suggest the Liver-Chip R1 has healthy rates of CYP metabolic activity, an essential hepatocyte function which plays an important role in liver health, disease, and drug toxicity.

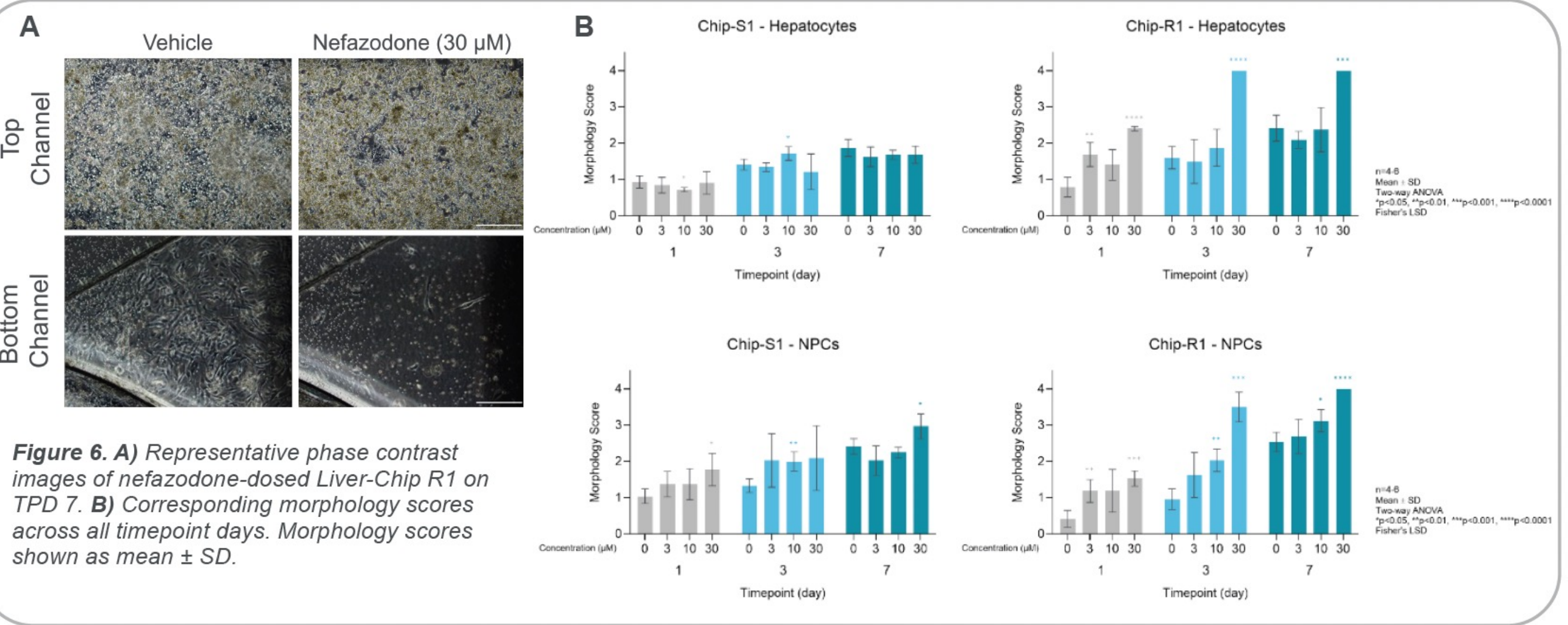


Liver-Chip R1 Toxicity Testing

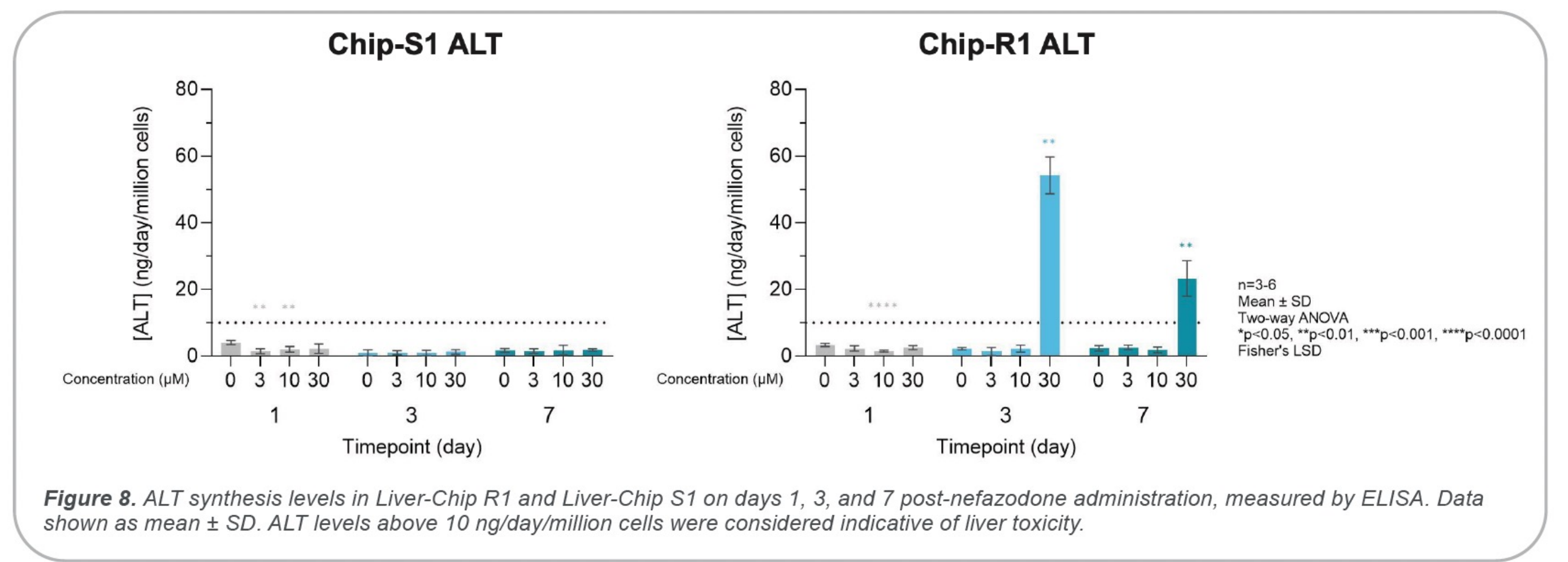
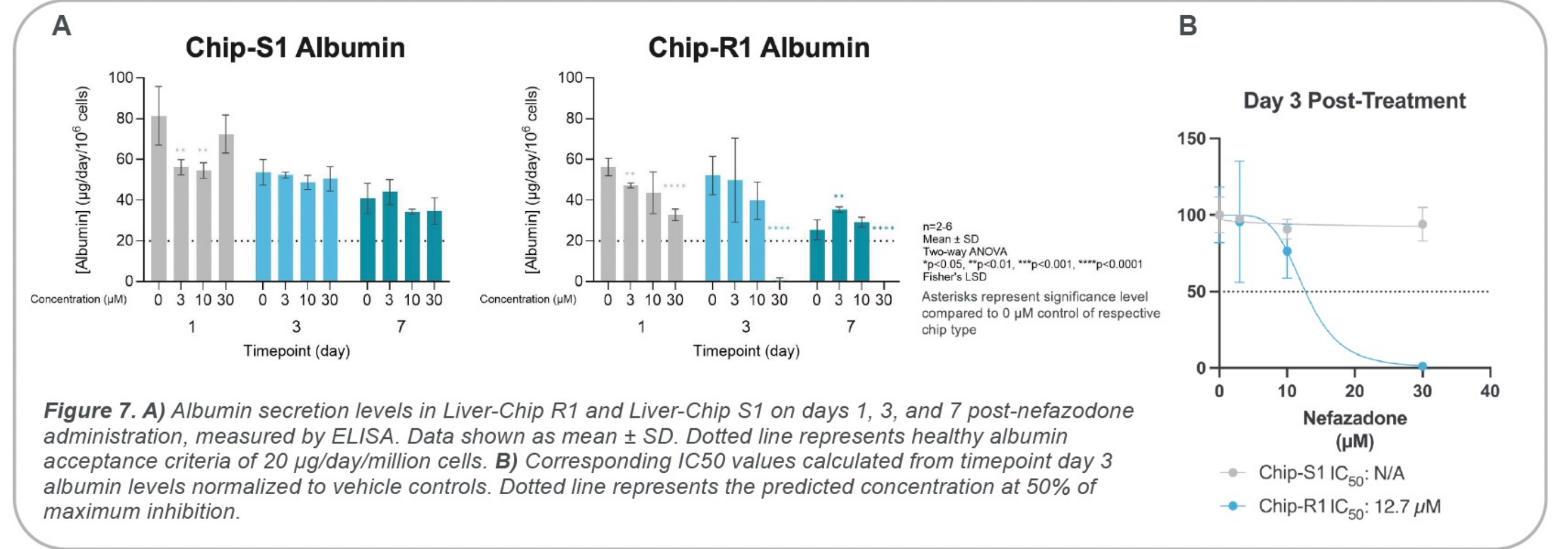
An One of the major advantages of Liver-Chip S1 is its predictive validity in detecting drug-induced liver injury (DILI) in hepatotoxic compounds (Ewart et al., 20222). To assess whether the Liver-Chip R1 offers similar or enhanced sensitivity, a comparative study was conducted using three compounds with different toxicity and PDMS absorption profiles.

Nefazodone, a highly hepatotoxic compound with high PDMS absorption, was administered at 3, 10, and 30 µM. Additionally, the structural analogs trovafloxacin and levofloxacin were tested, both compounds with minimal PDMS absorption. Trovafloxacin is a known hepatotoxic compound with a high ranking on the FDA and Garside DILI severity scales, while levofloxacin is less toxic (Garside et al., 20143). Trovafloxacin and levofloxacin were administered at 200 and 537 µM, respectively (data not shown).

The Chip-R1 demonstrated greater sensitivity in detecting DILI risk of the highly absorbing drug nefazodone, demonstrating the model's reduced drug absorption properties. When treated with 30 µM nefazodone at the protocol-recommended flow rate of 30 µL/h, the Liver-Chip R1 demonstrated significant signs of hepatotoxicity, with observable cell death via phase contrast imaging and morphology scoring (see Figure 6),



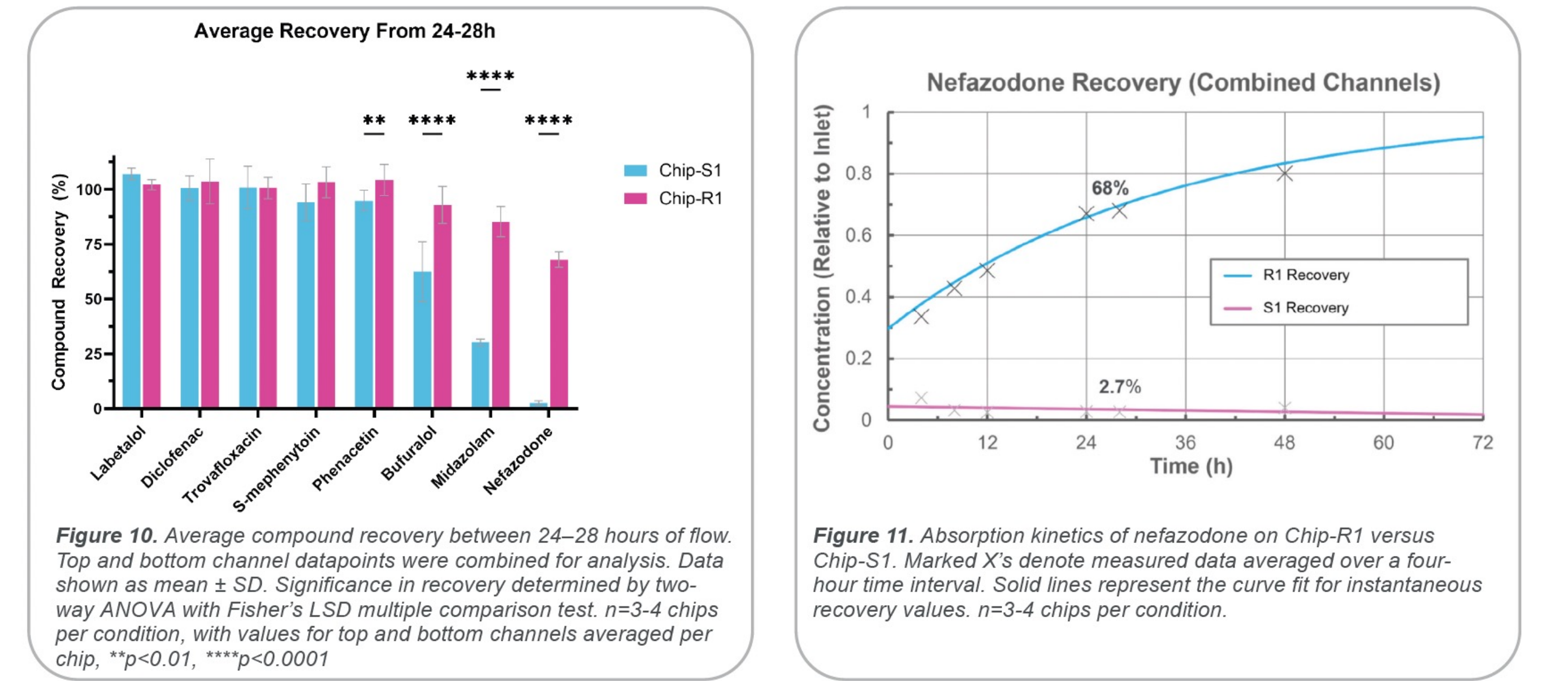
as well as a reduction in albumin production and an increase in alanine transaminase (ALT) release (see Figures 7 and 8) (Ewart et al., 20222). In contrast, the Liver-Chip S1 treated with the same concentration and flow rate of nefazodone showed minimal signs of DILI, with albumin, ALT production, and morphology scoring comparable to the vehicle-treated Liver-Chip S1. The increased sensitivity of the Chip-R1 allows for greater accuracy in predicting an IC50 value for nefazodone compared to the Chip-S1 consumable due to high absorption of nefazodone into PDMS at all concentrations tested (see Figure 7). This highlights the utility of the Chip-R1 consumable for studies aiming to predict the toxicity of compounds which may be highly liable to PDMS absorption.



Chip-R1 Drug Absorption Characterization

To characterize the ability of the Chip-R1 to minimize drug absorption, a panel of eight drugs was tested on both the Chip-R1 and Chip-S1. These drugs were selected for their relevance to hepatotoxicity or liver metabolic functions, as well as for their broad range of physicochemical properties. The drugs were administered to both channels of acellular Chip-S1 and Chip-R1 consumables at a flow rate of 30 µL/h. To assess compound recovery, effluent was collected from Pod reservoirs (inlets and outlets) for the 24–28 hour exposure interval. Drug recovery rates were calculated as the average ratio of Pod outlet concentration to Pod inlet concentration during this four-hour interval (see Figure 10).

Out of the eight drugs tested, three demonstrated high absorption on Chip-S1: nefazodone, bufuralol, and midazolam. These drugs would be predicted to have a significant risk of PDMS absorption based on their relatively high hydrophobicity, low molecular weight, and/or low topological polar surface area. In contrast, Chip-R1 showed a significantly improved recovery profile compared to Chip-S1:



Conclusion

The Chip-R1 Rigid Chip offers several advantages over the Chip-S1 Stretchable Chip, such as low drug-absorbing materials, a thinner, pre-activated membrane with smaller diameter pores, and a reduced vascular channel height which allows for an increase in maximum shear stress exposure. These enhancements make the Chip-R1 (and associated Pod-2) an ideal platform for studying small-molecule drug interactions with tissues that do not require stretch.

Results from the above equivalency study show that the Liver-Chip R1 replicates critical aspects of human liver physiology and serves as a biologically relevant model for assessing DILI. Comparative studies show that the Liver-Chip R1 maintains essential liver functionality, such as albumin production and metabolic activity, while improving sensitivity to hepatotoxic compounds prone to PDMS absorption. These findings suggest that the Liver-Chip R1 provides a more suitable model for evaluating the DILI risk of lipophilic small molecules.

References

- Deloitte. Pharma R&D return on investment falls in post-pandemic market. Deloitte. (2023, January 6). <https://www.deloitte.com/uk/en/about/press-room/pharma-r-d-return-on-investment-falls-in-post-pandemic-market.html>
- Ewart, L., Apostolou, A., Briggs, S.A. et al. (2022). Performance assessment and economic analysis of a human Liver-Chip for predictive toxicology. *Commun Med* 2, 154. <https://doi.org/10.1038/s43856-022-00209-1>
- Garside, H., Marcoe, K.F., Chesnut-Speelman, J. et al. (2014). Evaluation of the use of imaging parameters for the detection of compound-induced hepatotoxicity in 384-well cultures of HepG2 cells and cryopreserved primary human hepatocytes. *Toxicology in Vitro*, 28(2), 171–181. <https://doi.org/10.1016/j.tiv.2013.10.015>
- Mullard, A. Parsing clinical success. *Nature Reviews Drug Discovery*, 27(1), 1–2. <https://doi.org/10.1038/nrd.2017.101>