ARTICLE IN REVIEW:

TruVivo™: An all-human, stable, longterm culture system for primary human hepatocytes

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TITLE: The morphology, functionality, and longevity of a novel all-human hepatic cell-based tri-culture system

AUTHOR(S): Weaver JR, Odanga JJ, Wolf KK, Piekos S, Biven M, Taub M, et al.

STUDY DESIGN: Benchtop study *(in vitro)*

SUMMARY: Conventional *in vitro* models used to assess drug metabolism and toxicological effects often lack phenotypic and functional stability long term and can be inconvenient or difficult to use. TruVivo™, a novel all-human cell-based hepatic tri-culture system, addresses these limitations by combining cryopreserved primary human hepatocytes (PHH) with primary human feeder cells in a convenient, two-dimensional platform. PHH used in TruVivo were successfully cultured for 42 days *in vitro*, retaining their morphological characteristics and self-assembling into distinct hepatic microcolonies with formation of bile canaliculi and intercellular gap and tight junctions, mimicking the native hepatic architecture. Additionally, the PHH in TruVivo stained positive for key hepatic markers including albumin, CYP1A2, and CK18. To assess functionality, albumin and urea secretion, as well as CYP activity, were compared between TruVivo and sandwich monoculture (SC). On Day 14, TruVivo produced significantly higher levels of albumin (36.9 ± 0.6 μg/day/10^6 PHH) and urea (44.4 ± 1.0 μg/day/10^6 PHH) compared to SC (11.5 ± 4.5 μg/day/10^6 PHH and 9.7 ± 0.7 μg/day/10^6 PHH, respectively; p≤0.001 for each), which was stable through 22 days. CYP activity, induced with either omeprazole, CITCO, or rifampicin, was significantly higher than uninduced levels in both TruVivo and SC. However, the induced CYP activity levels were significantly higher in TruVivo versus SC, specifically for CYP2B6 (1171 ± 253 vs. 123 ± 80 pmol/min/10^6 PHH, respectively) and CYP3A4 (25,300 ± 3891 vs. 15,930 ± 1842 pmol/min/10^6 PHH, respectively; p≤0.001 for each), which was stable through 14 days in culture in TruVivo. Additionally, TruVivo showed formation of both phase 1 and phase 2 metabolites after exposure to 7-ethoxycoumarin, a probe substrate, that was stable through two weeks in culture. Collectively, these data demonstrate higher PHH functionality with TruVivo compared to SC that is stable over time. These results support TruVivo as an ideal alternative to conventional hepatic *in vitro* models because it represents "a [human] species and physiologically relevant, functionally stable, and convenient platform for pharmacological and toxicological applications in drug development and risk assessment," such as metabolic clearance of low-turnover compounds.

TruVivo maintained PHH morphology up to 42 days

PHH cultured in TruVivo retain their morphological characteristics for at least 42 days. Extensive bile canaliculi and tight junctions formed early, then remained stable and functional throughout the culture period.

TruVivo retained greater PHH functionality than SC

On Day 14, albumin and urea secretion was significantly higher in TruVivo compared to SC (p≤0.001). Induced CYP1A2, CYP2B6, and CYP3A4 activity was significantly greater in TruVivo than SC (p≤0.001). Induced CYP levels for both TruVivo and SC were significantly increased over uninduced, with similar uninduced levels between the groups. In TruVivo, CYP activity remained consistent and stable over the 14-day culture period (Fig. 1).

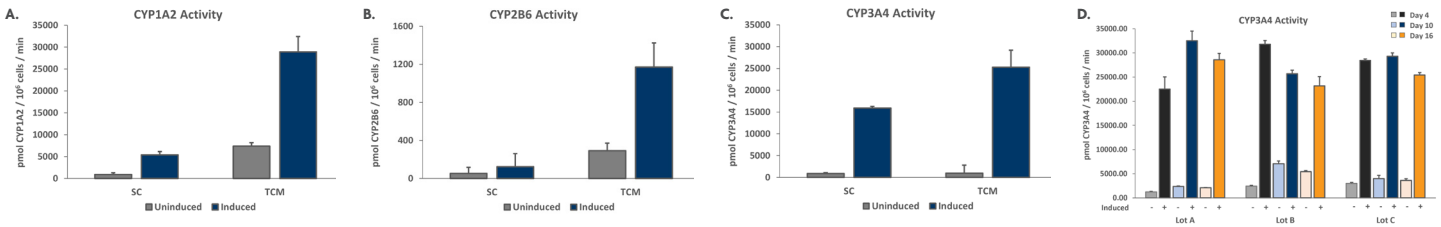
Stable generation of phase 1 and 2 metabolites

Following treatment with 7-ethoxycoumarin, a probe substrate, PHH in TruVivo generated both Phase 1 and subsequent conversion to Phase 2 metabolites, the ratio of which was stable through the 14-day culture period.

Reference

1. Weaver, J. R., Odanga, J. J., Wolf, K. K., Piekos, S., Biven, M., Taub, M., ... & LeCluyse, E. L. (2022). The morphology, functionality, and longevity of a novel all human hepatic cell-based tri-culture system. Toxicology in Vitro, 105504. https://doi.org/10.1016/j.tiv.2022.105504

CYP activity was greater in TruVivo compared to sandwich monoculture



(Fig. 1) Induced activity levels of **(A)** CYP1A2, **(B)** CYP2B6 and **(C)** CYP3A4 were measured in SC and TruVivo (TCM), showing significantly higher CYP activity with TruVivo than SC on Day 4. CYP1A2, CYP2B6, and CYP3A4 were induced with omeprazole, CITCO, or rifampicin for 48 hours before measuring enzyme activities. **(D)** Three different donor lots of PHH were cultured in TruVivo and uninduced (light shaded bars) and induced (dark shaded bars) CYP3A4 activity levels were measured on day 4, 10, and 16. *** p≤0.001 versus uninduced samples at the same time point. Mean ± SD. These results indicate retention of metabolic activity in PHH cultured in TruVivo that is stable over time and significantly greater than SC. Figure adapted with permission under an open access license.[1]

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