

ABSTRACT

Primary hepatocytes are essential for ADME and DMPK studies, but their rapid functional loss in 2D culture limits assessment of drug metabolism and toxicity. Here, we developed a simple 3D spheroid model using only primary hepatocytes, enabling rapid and reproducible formation of stable microtissues. The model preserves key drug-metabolizing enzyme activities (e.g., CYP450s), supporting evaluation of metabolic stability, enzyme induction, and drug-drug interactions. Its long-term stability allows assessment of chronic exposure and delayed hepatotoxicity. We further established a 3D non-alcoholic steatohepatitis (NASH) model that recapitulates disease-associated metabolic alterations, enabling compound screening and efficacy evaluation. This scalable and physiologically relevant platform provides a practical tool for integrated ADME/DMPK studies and disease modeling.

CONCLUSIONS

Primary hepatocytes rapidly self-assemble into uniform 3D spheroids under optimized conditions. The spheroids maintain long-term viability, liver-specific functions, and drug-metabolizing enzyme activity. Upon NASH induction, they recapitulate key disease features, including steatosis, fibrosis, and metabolic dysregulation. This platform supports integrated ADME/DMPK and pharmacology studies, enabling assessment of intrinsic clearance, enzyme induction, drug-drug interactions, hepatotoxicity, and therapeutic efficacy via multi-endpoint readouts.

PATENTS

- Qu M, Li X, Huang B, Patent Application No.: CN202510203353.X.
- Qu M, Li X, Chen S, Huang B, Patent Application No.: CN202410307704.7.

RESULTS

1. Primary hepatic spheroid allows long-term culture and maintains cell viability, metabolic activity, and key liver functions

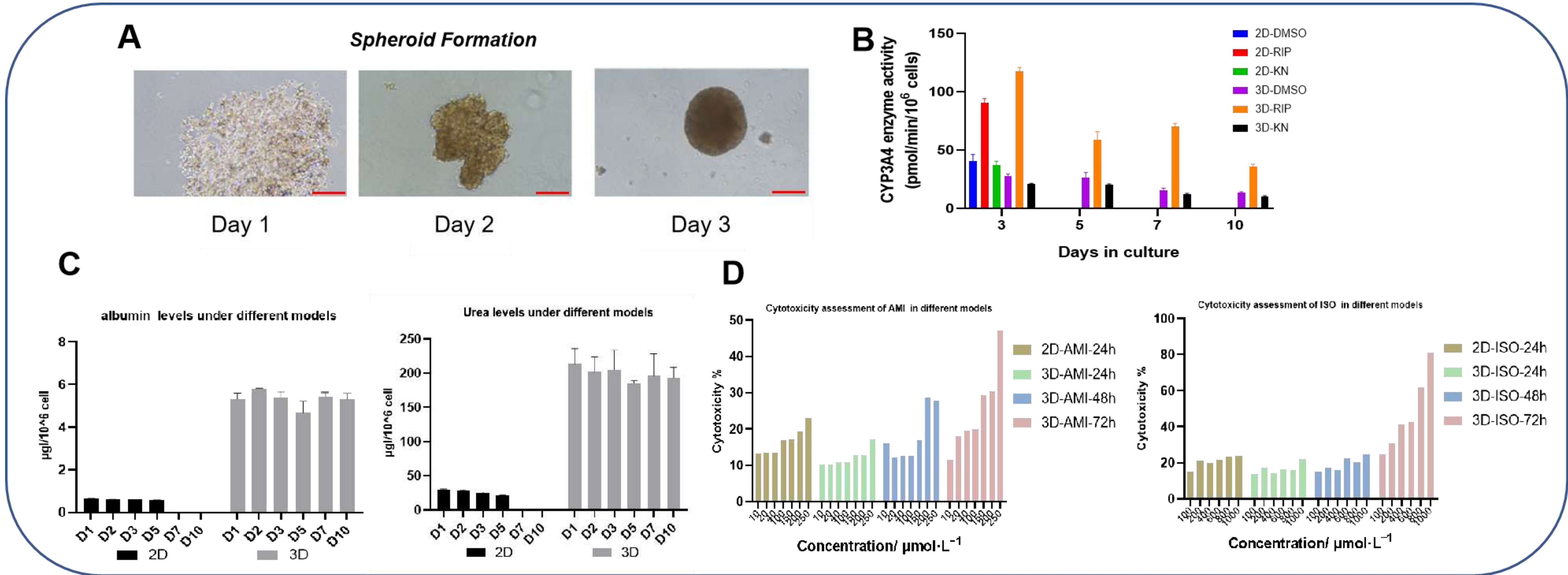


Fig. 1 Advantages and workflow of the primary hepatocyte 3D model. (A) Spheroid formation process using cynomolgus monkey primary hepatocytes via a two-step culture method, showing rapid self-aggregation from Day 1 to Day 3. (B) CYP3A4 activity (pmol/min/10⁶ cells) was measured in hepatocytes cultured in 2D or 3D at days 3, 5, 7, and 10 following treatment with DMSO, rifampicin (RIF), or ketoconazole (KN). RIF strongly induced CYP3A4 activity in both systems, with a greater effect in 3D, especially at day 3, though activity declined over time. DMSO showed moderate (2D) to low but stable (3D) basal levels, while KN remained low throughout. Overall, 3D culture better preserved CYP3A4 function than 2D. (C) The 3D model sustains liver-specific functions, including stable albumin and urea secretion over an extended period. (D) The model enables detection of both acute (AMI) and chronic (ISO) hepatotoxicity, demonstrating time-dependent and dose-responsive effects.

2. Hepatocytes-induced non-alcoholic steatohepatitis (NASH) spheroid is suitable for drug candidates evaluation

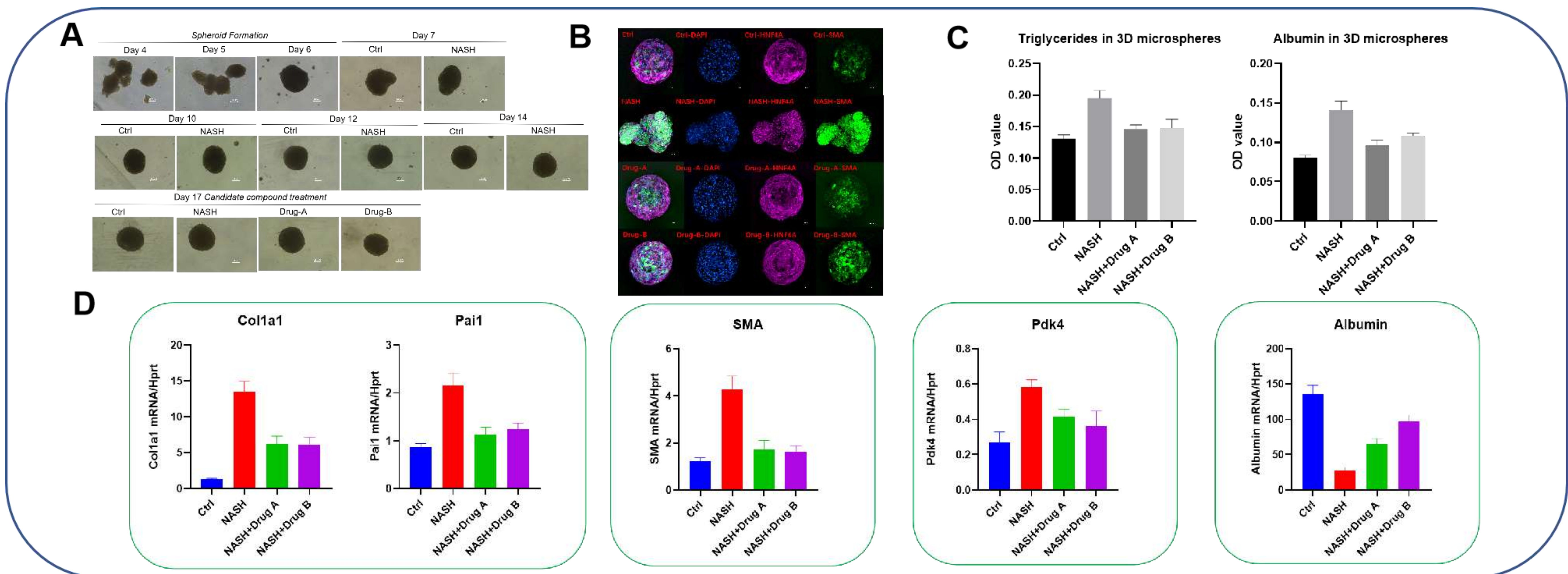


Fig.2 Advantages of 3D model in toxicity test and disease modeling. (A) Formation of 3D NASH spheroids using CD-1 mouse primary hepatocytes. Spheroids were cultured for 6 days to allow rapid stabilization, followed by addition of 100 µL induction medium containing monosaccharides, fatty acids, and BSA. Thereafter, 50 µL of medium was replaced every two days with fresh induction medium. After 7 days of induction, spheroids were further stimulated with 10 ng/mL LPS for an additional 3 days. (B) NASH spheroids display increased fibrosis markers compared with healthy controls, while treatment with candidate drugs attenuates this phenotype, as shown by imaging and marker expression. (C) NASH spheroids exhibit elevated triglyceride accumulation and altered albumin secretion, both of which are partially restored upon treatment with Drug A (Selonsertib) and Drug B (Obeticholic acid). (D) qPCR analysis shows significant upregulation of NASH-associated genes (e.g., Col1a1, Pai1, SMA, Pdk4) and downregulation of liver function markers (e.g., Albumin) compared to controls; these changes are mitigated following drug treatment.