

Title: Comparative Assessment of Advanced hiPSC-Derived Liver Organoid Protocols for Metabolic Disease Modeling

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Abstract

Liver organoids derived from human induced pluripotent stem cells (hiPSCs) offer a transformative platform for modeling hepatic physiology and disease, overcoming key limitations of conventional 2D cultures and spheroid models, which insufficiently replicate the liver's multicellular organization, functional maturation, and secretion of essential metabolic products such as lipoproteins. The urgent need for robust, standardized organoid systems is underscored by the global burden of non-alcoholic fatty liver disease (NAFLD, MASLD)—a condition driven by hepatic lipid accumulation and metabolic dysfunction that remains difficult to recapitulate in vitro. This study systematically compares two advanced organoid generation protocols: the Stemcell Technologies HepatiCult™ method, built upon in-house differentiated hiPSC-derived hepatocyte-like cells (HLCs), and the Takanori Takebe vascularized protocol, implemented with minor workflow modifications. Organoids are generated in parallel and matured under controlled conditions, with the primary aim of identifying protocol-dependent differences in hepatic maturation and functional capacity. Key methods include analysis of cellular composition and assessment of lipoprotein secretion as a direct measure of mature liver metabolic output. Advanced downstream analysis will employ single-cell RNA sequencing to characterize cellular heterogeneity, lineage allocation, and gene expression dynamics during organoid development and differentiation. Initial results establish pre-differentiation cellular states for protocol comparison; future experiments will introduce patient-derived adipocyte-conditioned media to simulate paracrine interactions central to NAFLD pathogenesis and validate disease modeling capability. The overall goal is to identify culture and protocol variables that maximize hepatic maturation and disease modeling accuracy, providing critical insights for translational research, drug development, and organoid-based liver platforms.