**3D bioengineered liver for the study of acute and chronic hepatic damage**

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The liver, a vital organ, faces acute and chronic insults that disrupt its normal function. Acute damage, caused by toxins or infections, triggers inflammation and necrosis. Chronic insults, such as alcohol abuse or viral hepatitis, lead to fibrosis, cirrhosis, and hepatocellular carcinoma, posing significant clinical challenges. Fibrosis is a hallmark of liver damage driven by the activation of hepatic stellate cells (HSCs). Understanding the mechanisms underlying acute and chronic liver damage is crucial for developing effective treatments. Traditional liver models face several limitations. 2D cultures cannot maintain liver phenotype and functions for extended periods, making it difficult to model chronic exposure. Additionally, replicating fibrosis in 2D cultures is challenging due to HSC activation on plastic or glass surfaces. As a result, 3D models have emerged as a more physiologically relevant cellular microenvironment for investigating disease progression, identifying potential therapeutic targets, and developing new drugs.

We developed a 3D liver using human hepatocytes (HepaRG), HSCs (LX-2), and monocytes (THP-1). The cells were encapsulated in a mixture of gelatin methacryloyl and carboxymethyl cellulose methacrylate, and lithium phenyl(2,4,6-trimethylbenzoyl)phosphonate as a photo-initiator. The 3D livers were kept in culture for up to 30 days in serum-free medium. They were challenged with acetaminophen and LPS (APAP-LPS), known hepatotoxic compounds, to recreate the pathophysiological phenotype of liver damage in vitro. Dexamethasone was used as an anti-inflammatory drug to test the ability of 3D livers to predict drug efficacy.

Extensive liver damage characterized by hepatic stellate cell (HSC) activation and proliferation was observed upon challenge with APAP-LPS. In vivo, these cells exhibited the myofibroblast phenotype typical of activated HSCs. Additionally, impaired gene expression of hepatocyte functionality markers was observed. The transition from monocytes to proinflammatory cytokine-releasing macrophages measured the inflammation level. Notably, dexamethasone demonstrated potent beneficial effects, reducing hepatocyte damage, inhibiting HSC activation, and decreasing collagen production. These results were observed in both acute (high APAP-LPS concentration/3 days) and chronic (low APAP-LPS concentration/30 days) models.

The 3D model presented here demonstrates its value as a versatile platform for drug screening in both acute and chronic liver damage scenarios. Its ability to reproduce critical features of liver pathophysiology, including hepatocyte functionality impairment, HSC activation, and inflammation, makes it a valuable tool for studying liver diseases and evaluating potential therapeutic interventions. Furthermore, the adaptability of this model for high-throughput screening provides an opportunity to accelerate the drug discovery process and improve patient outcomes in liver damage-related conditions.

**Disclosures**

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