DEVELOPMENT OF A NOVEL HUMAN 3D IN VITRO MODEL FOR EVALUATING NEW ANTI-FIBROTIC DRUGS

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Abstract

The discovery and development of anti-fibrotic therapies remains heavily reliant on animal testing. There is an urgent need to develop robust and relevant in vitro models to support the identification and preclinical evaluation of potential new anti-fibrotic drugs. Herein, we present a novel human 3D liver co-culture model using our proprietary ORGANDOT® platform. These liver ORGANDOT cultures (human hepatocytes, Kupffer cells and stellates) not only maintain viability and functionality for up to four weeks in culture, but can also be treated with TGF-β to induce a fibrotic phenotype. The TGF-β treated ORGANDOT cultures show a decrease in hepatocyte function and a concomitant increase in fibrogenic gene expression (COL1A1, SPP1, TIMP2). Furthermore, co-administration of an AKT inhibitor was able to completely prevent these fibrotic changes and rescue the functionality of the cultures. These data demonstrate the potential utility of this in vitro model for testing the efficacy of new anti-fibrotic drug candidates for research into the mechanisms of liver fibrosis and fibrosis reversal.

Methods

Isolation of Human Hepatic Stellate Cells

Macropositively enriched human liver tissue was used for the isolation of stellate cells. All samples were processed with informed consent. Stellate cells were isolated in-house according to established protocols. In brief, the tissue was minced and digested by incubating with 0.02% pronase and 0.02% collagenase B (Roche) for 60 minutes at 37°C in a shaking incubator at 200 rpm. The cell suspension was then filtered, and the debris cells pelleted using a discontinuous (pH 6.1) gradient (Sigma). The purified stellate cells were then expanded in culture in DMEM-high glucose supplemented with 10% FBS and 1x Antibiotic-Antimycotic (Sigma) for up to 28 days.

Creation of Liver ORGANDOT Cultures

Cryopreserved human hepatocytes were obtained from Tangle Research Laboratories (UK). Cryopreserved human Kupffer cells were obtained from Life Technologies. On the day of ORGANDOT creation, hepatocytes and Kupffer cells were thawed according to manufacturer's protocols. Hepatocytes were seeded at 2 x 10⁶ for 8 hours before being transferred to 10% Hepatocyte maintenance medium (MM250). Kupffer cells were seeded at 5 x 10⁵ for 5 minutes and were re-seeded in Life Technologies' medium (medium supplemented with BSA, Hepes, L-glutamine and 1x Antibiotic-Antimycotic) for 20 minutes. The cell suspension was then transferred to 10% Hepatocyte maintenance medium (MM250) and were maintained for 4 hours at 37°C, 5% CO2, with renewal of the basolateral medium every 48 hours. The ORGANDOT platform was then assembled on a multi-layered semi-permeable membrane, and the cultures were cultured at an air-liquid interface for 7 days.

Results

Figure 1: Maintenance of viability and functionality of liver ORGANDOT c-cultures

Figure 2: Kupffer cell functionality is maintained in liver ORGANDOT c-cultures

Figure 3: Liver ORGANDOT c-cultures show an increase in fibrogenic gene expression that is prevented by the AKT inhibitor SB525334

Figure 4: TGF-β1 treated liver ORGANDOT c-cultures show an increase in collagen deposition that is prevented by the AKT inhibitor SB525334

Figure 5: Collagen I (normalised copy number)

Conclusions

- Liver ORGANDOT cultures (human hepatocytes, Kupffer cells and stellates) maintain viability and functionality for up to 28 days in culture.
- Exposure to TGF-β1, a central regulator in liver fibrosis, induced a fibrotic phenotype with an increase in hyaluronic acid accumulation and Kupffer cell activation.
- Co-administration of the AKT inhibitor, SB525334, was able to prevent these fibrotic changes and rescue the functionality of the ORGANDOT cultures.
- These data demonstrate the potential utility of this novel 3D in vitro model for evaluating new anti-fibrotic drugs or for studying the mechanism of fibrosis induction and hepatic stellate cell activation.
- These data also extend the use of the ORGANDOT platform to additional disease models, which now include diabetes, oncology, and acromegaly, here, liver fibrosis.

References


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