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| ***In-situ* Fabrication of Human Liver Spheroids with Biomimetic Nanoscaffolds** |
| Lina Wu, Driton Vllasaliu and Bahijja Tolulope Raimi-Abraham |
| School of Cancer and Pharmaceutical Sciences, King’s College London, London SE1 9NH, UK |
| **Background:** Recent U.S. legislation has eliminated the mandatory animal testing requirement for drugs in development, further driving the demand for innovative animal-free approaches. Traditionally, animal models have been used for preclinical testing of drug-induced liver injury (DILI), but ongoing debates have highlighted the importance and need for alternative non-animal-based models. 3D cell culture models, such as spheroids and organoids, have gained attention for their ability to mimic in vivo environments. Spheroids are preferred for high throughput drug toxicity testing due to their simpler production methods and higher reproducibility compared to organoids. However, common scaffold-free spheroids suffer from decreased cell viability in long-term culture. To overcome this limitation, polymeric nanoscaffolds can be used to engineer human liver spheroids by providing structural support and creating a favourable microenvironment for spheroid growth and function. The goal of this study was to fabricate human liver spheroids with biomimetic polymeric nanoscaffolds for preclinical screening for liver toxicity. Polycaprolactone (PCL) was used to generate nanoscaffolds by electrospinning and spheroids were fabricated by hanging drop method. The effect of nanoscaffolds on the size, which would affect the cell viability, and albumin secretion, which is a critical indicator of liver function, of spheroids was investigated. Furthermore, a dose-dependent acetaminophen toxicity test was performed to verify the drug metabolism capability of our models. |
| **Methods:** The nanoscaffolds were generated by the electrospinning method and cryogrinding. The spheroids were generated by hanging drop method and three samples were fabricated: spheroids without nanoscaffolds, spheroids with 0.005% w/v and 0.01% w/v nanoscaffolds. The morphology of nanofibres and spheroids were evaluated using scanning electron microscopy (SEM) and phase contrast microscope respectively and the sizes were analysed by Image J. The cell viability was evaluated by live-dead staining and an ATP-based assay on day 3, 5, 7, 9 and 11. The albumin secretion was tested by ELISA. A dose-dependent acetaminophen toxicity test was performed on day 5 on 2D cell culture model, spheroids without nanoscaffolds, spheroids with 0.005% w/v and 0.01% w/v nanoscaffolds and the IC 50 value was calculated. Data expressed are representative of at least three independent experiments (n = 3) in triplicate and represented as mean ± standard error. A student t-test was used to discern the statistical difference between the two groups. A probability value (p) of less than 0.05 was considered to be statistically significant. |
| **Results:** The results showed that the diameters of spheroids could remain lower than 400 μm to avoid central necrosis and improved cell viability was observed in nanoscaffold-based spheroids. The spheroids with nanoscaffolds were able to synthesize and secrete more albumin than scaffold-free spheroids on day 5 and 7, indicating liver-specific functionality. In addition, the spheroids with nanoscaffolds were more sensitive to acetaminophen compared to 2D monolayer cultures and scaffold-free 3D spheroids with IC 50 values in > 5,000 μM, 702.2 μM, 105.8 μM, 537.3 μM for 2D cultured cells, spheroids without nanoscaffolds, spheroids with 0.005% w/v and 0.01% w/v nanoscaffolds respectively. |
| **Conclusions:** These findings suggest that our scaffold-based spheroid models have great potential as in vitro liver models for DILI. Future work will focus on the prediction of liver toxicity of candidate drugs. |