Stiftung zur Förderung der Erforschung von Ersatz- und Ergänzungsmethoden zur Einschränkung von Tierversuchen

Project

Development of a 3D flow through model for long-term culture of polarized hepatocytes (-like cells) *in vitro*

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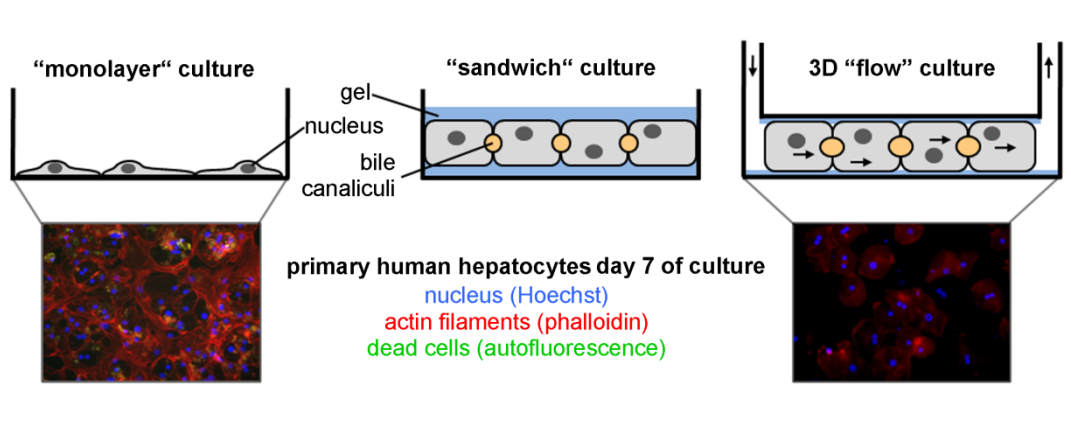
The aim of this project is the development of a standardized and continuously available *in vitro* model to measure long-term cellular responses, e.g. chronic inflammation and toxicity, drug-drug interactions or CYP induction in humans. To prevent species-dependent false-positive or false-negative results primary human hepatocytes shall be used. A 3-dimensional arrangement of the primary hepatocytes will allow long-term culture of the cells.

Therefore, the cells will be cultured in a 3D-flow through model ("μ-slides"), to allow formation of bile canaliculi and their emptying. The associated continuous exchange of medium offers an optimal supply of nutrients for the cells as well as the controlled application of test substances, similar to cultures in a bioreactor. However, to fill a "μ-slide" significantly less cells are needed, which enables screenings using the limited available primary human hepatocytes. Due to the transparent design of the "μ-slides" morphologic changes of the cells during culture can be documented. Furthermore, after finishing the experiments the cells can be stained inside the "μ-slide".

Besides primary human hepatocytes, we want to test the system with two different human hepatocyte-like cells with good metabolic characteristics, in order to allow continuous availability of the system. The different cell types will be analyzed for the morphologic and functional changes during short- and long-term cultures in the system. Main focus will be on their metabolic competence. For the internal validation of the system, the obtained data will be compared to own *in vitro* data from 2D-cultures as well as *in vivo* data from public databases.

Summarizing, we expect that the newly developed system leads to a more effective use of primary human hepatocytes and thus reduces species-dependent false-positive or false-negative results. Furthermore, we expect that the system will give information on secondary cellular mechanisms and thus improves the safety of newly developed substances.



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