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3D-BIOPRINTING OF HEPG2-CELLS AND CONSTRUCTION OF A LIVER-ON-A-CHIP SYSTEM AS A POTENTIAL SCREENING-DEVICE FOR DRUG-HEPATOTOXICITY

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BACKGROUND

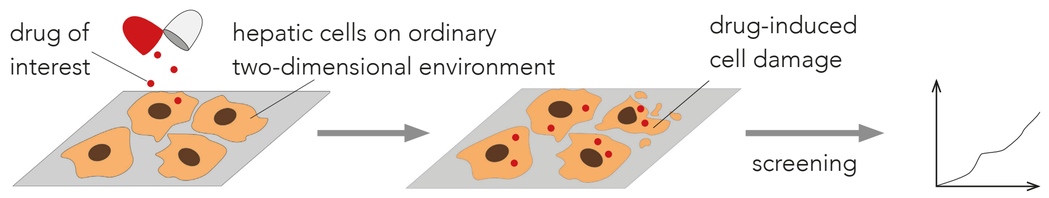
One of the main challenges regarding the safety evaluation of new pharmaceutical drugs is the screening for hepatic toxicity, which is done by standardized in-vitro, as well as in vivo-testing. In current in-vitro assays, the drug of interest is added to ordinary two-dimensional cultures of human hepatic cells.[1] One of the main problems in this approach however, is that a two-dimensional microenvironment does not resemble the original three-dimensional network of a real organ, resulting in important differences in cell-shape, behavior and metabolism.[2] Moreover, because of substantial differences between the hepatic metabolism of humans and laboratory-animals, about 50% of drug-induced liver injuries (DILI's) can not be sufficiently predicted by ordinary in-vivo models.[3] In humans, DILI's are therefore considered the main cause of toxicological concerns regarding newly approved as well as already approved medicinal products.

For those reasons new kinds of in-vitro assays are needed to evaluate the hepatotoxicity of future drug-candidates in a manner more applicable to the human metabolism

OBJECTIVES

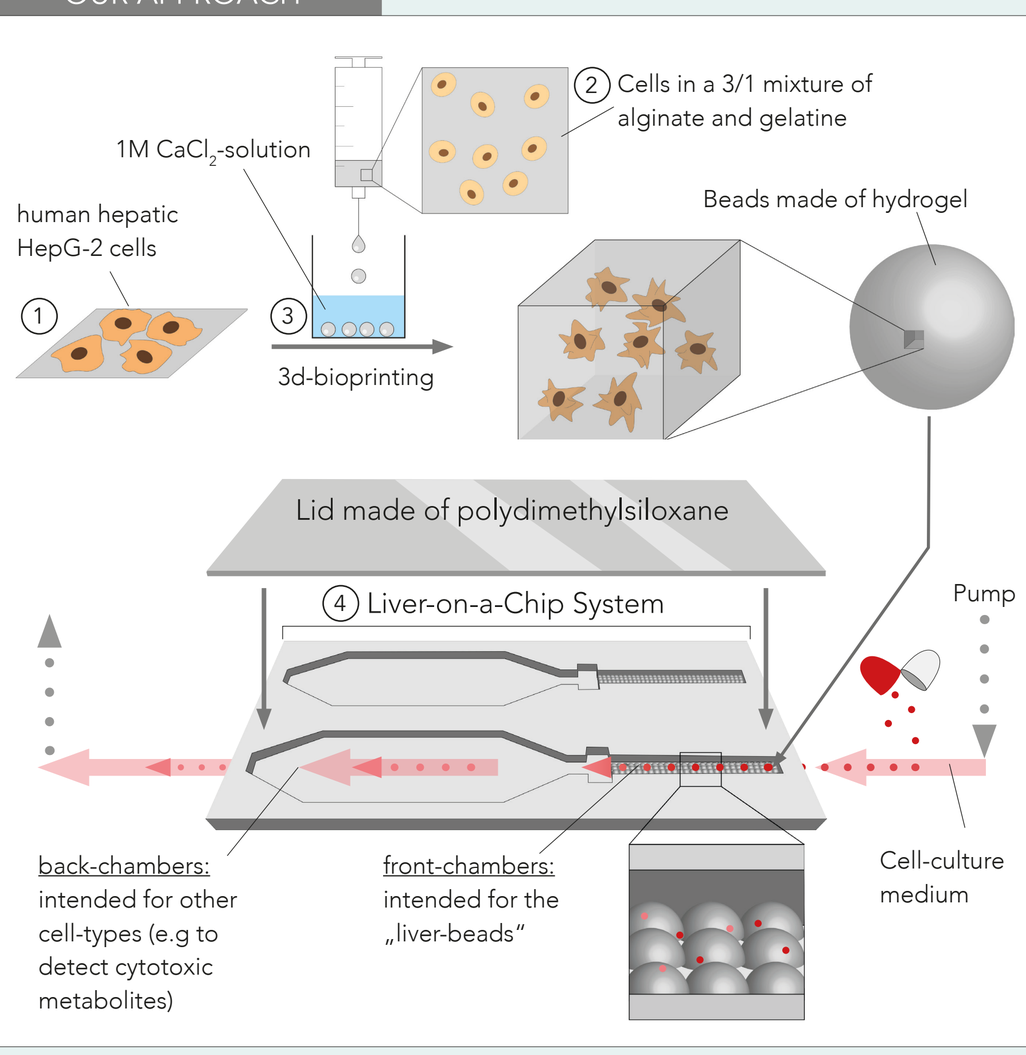
It has been shown that the behaviour of hepatic cells embedded in 3-d environments made of hydrogels that resemble many characteristics of their real physiological habitat is more comparable to in-vivo conditions.[4] Our goal was the construction of a so called „Liver-on-a-Chip System", where the cells of a human hepatic cell-line are embedded in spheroidal 3-d structures (beads) made of hydrogel, as a potentially more conclusive screening-device for hepatic drug-metabolism and toxicity.

REGULAR APPROACH



METHODS

OUR APPROACH



(1) HepG-2 cells are an affordable, easy to handle and widely used type of human liver-carcinoma cells, possesing many essential characteristics of primary hepatocytes. Hence they have already been used successfully in similar approaches.[4]

(2) To create a 3-dimesional extracellular environment, a mixture of alginate and gelatine was used, where the alginate mainly served for stability, whereas the gelatine served for cytocompatibility by enabeling cell-adhesion. To determine the most suitable composition, at first empty beads (blind-beads) containing different alginate/gelatine ratios were printed and characterized based on their size, circularity and mechanical properties (elastic modulus). Subsequently, the chosen hydrogel containing 1% alginate in a 3/1 ratio to gelatine, was mixed evenly with HepG-2 cells.

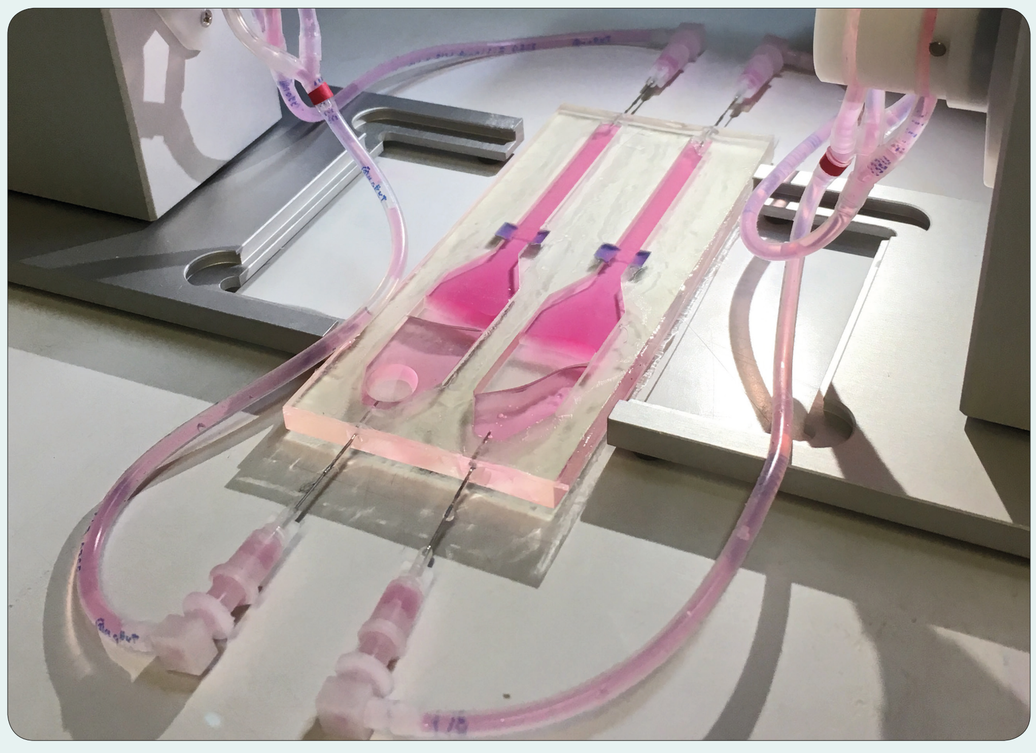
(3) The beads were printed with an injection pump using a cannula with 0,4 mm in diameter. For the droplets, to form stable spheroidal structures, the hydrogel mixture was dropped directly into a 1M solution of $CaCl_2$, in which it has been incubated for 15 min, enabeling crosslinking between alginate-strands and thus polymerization in a calcium dependant manner.

(4) The prototype of the chip-system was made out of epoxy resin using 3d-printing and was designed with four-chambers: two front-chambers to place the printed beads containing the hepatic-cells and two back-chambers where other cell-types might be placed e.g for the detection of cytotoxic metabolites resulting of hepatic metabolism. That way, one chamber-system can be used for toxicity-screening, while the other could serve as a negative control. By attaching the chip to a pump, a continuous fluidic system could be realized.

RESULTS AND DISCUSSION

The blind-beads had an average diameter of 2,075 mm, rangig from 1,655 up to 2,447 mm, which might be small enough to allow diffusion of nutrients even to the cells embedded in the center of the beads - though further research is needed to investigate this assumption. Also, all beads showed high levels of circularity near or equal to 1,000. Moreover, elasticity measurements revealed average values of elastic-modules between 2,38 and 4,26 kPa, corresponding with the elasticity of soft tissue. However further research is needed to investigate morphological and metabolic characteristics of the cells embedded in those structures, as well as differences to conventional 2d-cultures. As mentioned, the printing process with HepG2-cells was realized by using a hydrogel-mixture containing 1% alginate in a 3/1 ratio to gelatine. Blind-beads with that composition exhibited an average diameter of 1,967 mm and a circularity of 0,996.

Attaching the chip to a pump allowed the continuous flow of medium through the chambers. However further optimization is necessary. To be applicable as a screening device, the chip-system has to be made of a material high in biocompatibility. Furthermore a method has to be developed in which the beads can be placed in the chambers under sterile conditions - which may possibly require a different design.



SOURCES

[1] Soldatow et al. In vitro models for liver toxicity testing. Toxicol Res (Camb). 2012;2(1):23-39.
[2] Faulkner-Jones et. al (2015). Bioprinting of human pluripotent stem cells and their directed differentiation into hepatocyte-like cells for the generation of mini-livers in 3D. Biofabrication, 7(4), 044102.
[3] Olson, H. et al. (2000). Concordance of the Toxicity of Pharmaceuticals in Humans and in Animals. Regulatory Toxicology and Pharmacology, 32(1), 56–67
[4] Ramaiahgari, S. C. et al. (2014). A 3D in vitro model of differentiated HepG2 cell sphroids with improved liver-like properties for repeated dose high-throughput toxicity studies. Archives of Toxicology, 88(5), 1083–1095.