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Isolation and characterization of human hepatocytes and non-parenchymal liver cells

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University Medicine Berlin, Department for General, Visceral and Transplantation Surgery, led by Dr. Georg Damm (and Prof. Dr. Daniel Seehofer) have established a protocol for an uncomplicated isolation of primary human hepatocytes (PHH), Kupffer cells (KC), liver endothelial cells (LEC), and human Stellate cells (HSC) from human donor tissue. Liver cells were isolated from the tissue using a two-step EDTA/collagenase perfusion technique, followed by a separation of PHH and different non-parenchymal cell (NPC) fractions through Percoll density gradient centrifugation, an adherence separation step (KC) and magnetic activated cell sorting (HSC, LEC). All isolated cell fractions were identified by specific morphological and functional characteristics and were examined during a defined cultivation time.

The findings, which appear in the May 2015 issue of *Experimental Biology and Medicine*, provide a new simplified method for isolation and fractionation of human liver cells, which can be used to establish defined co-culture models reflecting the in vivo situation in the organ, e.g. for studies on drug toxicity or for development of disease models.

"PHH mono-cultures are considered to be the gold standard for the investigation of hepatic metabolism and toxicity of xenobiotics. However, detailed morphological and functional studies have demonstrated that these models are limited due to hepatocyte dedifferentiation and loss of functions within a few days. Reconstruction of the in vivo tissue architecture consisting of a 3D environment including NPC is a promising approach to solve some of these problems"

states Dr. Georg Damm. "In conclusion, we present a new method for simultaneous extraction of human PHH and different NPC populations from the same donor tissue. The cells were clearly identified and characterized on the basis of morphological properties, specific marker expression and functional analysis."

Future studies are needed to monitor the fate and the crosstalk of the isolated NPC in functional co-culture models and engineered micro-tissues. In this regard the isolated cells in the published isolation protocol form the basis for in vivo like physiological and patho-physiological liver models.

Dr. Steven R. Goodman, Editor-in-Chief of *Experimental Biology and Medicine*, said "Pfeiffer et al have provided a method for the simultaneous isolation of human PHH and distinct populations of NPC from the same human tissue. The value of this new method will be for the co-culture of hepatocytes and distinct NPC fractions which holds promise for the future of drug testing and liver tissue engineering."

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