Hepatology Snapshot

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Precision-cut liver slices: A tool to model the liver *ex vivo*

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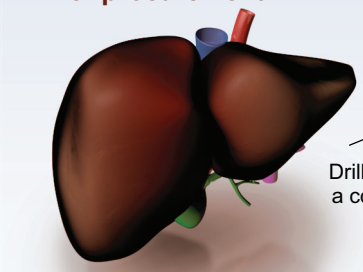
Preparation and culture of precision-cut liver slices (PCLS)

1. Liver procurement



Drilling a core

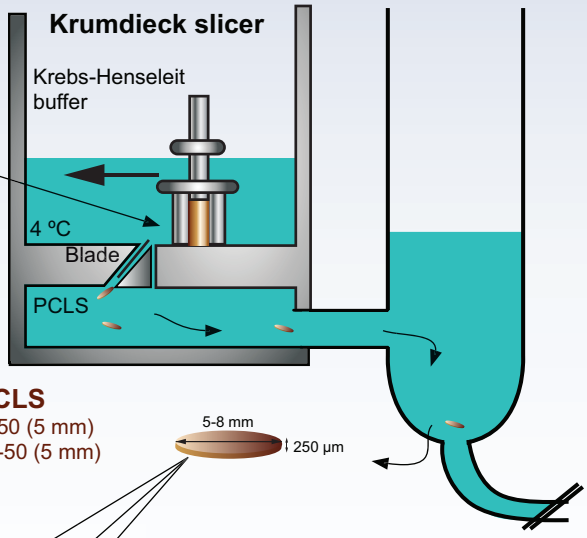
Transfer to Krumdieck slicer

Core Ø 5-8 mm

de Graaf, I.A.M. *et al.,* Nat Protoc, 2010
Fisher, R.L. *et al.,* Xenobiotica, 2013

2. Preparation of PCLS

de Graaf, I.A.M. *et al.,* Nat Protoc, 2010
Fisher, R.L. *et al.,* Xenobiotica, 2013



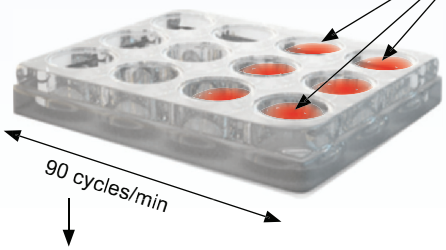
3. Number of PCLS

- Rat liver: 200-250 (5 mm)
- Mouse liver: 40-50 (5 mm)

4. Culture systems

- Submerged systems (well plates)
- Dynamic organ culture system

Olinga, P. *et al.*, J Pharm Toxic Meth, 1997
de Graaf, I.A.M. *et al.,* Nat Protoc, 2010
Fisher, R.L. *et al,* Xenobiotica, 2013



5. Culture of PCLS

- 12 well plates
- William Medium E supplemented with 25 mM glucose and 50 µg/ml gentamycin
- Under 95% O_2/5% CO_2
- At 37 °C

de Graaf, I.A.M. *et al.,* Nat Protoc, 2010
Fisher, R.L. *et al.,* Xenobiotica, 2013

Studies

PCLS

Culture of PCLS — Fisher, R.L. *et al.*, Xenobiotica, 2013

Studies	Results	Sp	[Ref.]
Long-term culture	Metabolic capacity stable for 3 days (phase II enzymes more stable, than phase I enzymes)	h	Ioannides, C. *et al.*, Xenobiotica, 2013

Disease

Fibrosis (induced *ex vivo*) — Westra, I.M. *et al.*, Xenobiotica, 2013

Studies	Model compound	Results	Sp	[Ref.]
Model of fibrogenesis	Ethanol	Col1A1 ↑	r	Schaffert, C.S. *et al.*, Am J Physiol-Gastr L., 2010
	Acetaldehyde	HYP ↑	r	Guo, Y. *et al.*, J Med Food, 2012
	CCl_4	HSP47 ↑	r, h	van Bovenkamp, M. *et al.*, Toxicol Sci, 2005 van Bovenkamp, M. *et al.*, Chem Biol Interact, 2006

Fibrosis (induced *in vivo*) — Westra, I.M. *et al.*, Xenobiotica, 2013

Studies	*In vivo* model	Compound	Results	Sp	[Ref.]
Anti-fibrotic activity	Bile duct ligation	Gliotoxin-M6P-HSA	Desmin ↓	r	Hagens W.I. *et al.*, Liver Int, 2008
	Bile duct ligation	Gleevec	Pcol1A1 ↓	r	van Bovenkamp, M. *et al.*, J Hepatol, 2006

ADMET

Drug metabolism — Lake, B.G. *et al.*, Xenobiotica, 2013

Studies	Compound	Results	Sp	[Ref.]
Metabolic pathway	Precocene I	Metabolic pathway inhibited by specific inhibitors	r	Ly, V.T. *et al.*, Chem Biol Interact, 2011
Metabolic rates	i.e. quinidine and testosterone	*In vivo* metabolic clearance predicted	r	de Graaf, I.A.M. *et al.*, Drug Metab Dispo, 2006
Induction of metabolism	Phenobarbital, β-naphthoflavone, and rifampicin	CAR, AhR and PXR pathways were successfully studied	h	Olinga, P. *et al.*, Eur J Pharm Sci, 2008

Drug toxicity — Lake, B.G. *et al.*, Xenobiotica, 2013

Studies	Compound	Results	Sp	[Ref.]
Predict *in vivo* hepatotoxicity	Paracetamol and CCl_4	Predicted toxicity and pathology observed *in vivo*	r	Elferink, M.G.L. *et al.*, Toxicol Appl Pharmacol, 2008

Drug transport — de Graaf, I.A.M. *et al.*, Expert Opin Drug Metab Toxicol 2007

Studies	Compound	Results	Sp	[Ref.]
Regulation of drug transport	LPS	Rat results in line with *in vivo* (NTCP and MRP2 ↓)	r, h	Elferink, M.G.L. *et al.*, Am J Physiol Gastrointest Liver Physiol, 2004

Table 1. Studies performed in PCLS. Sp, species; h, human; r, rat; HSP47, heat-shock protein 47; Col1A1, collagen 1α(I); Pcol1A1, procollagen 1α(I)/mRNA; HYP, hydroxyproline; M6P-HSA, mannose-6-phosphate-human serum albumin; CAR, constitutive androstane receptor; AhR, aryl hydrocarbon receptor; PXR, pregnane X receptor; NTCP, Na^+-taurocholate co-transporting polypeptide; MRP2, multidrug resistance associated protein 2.

Future avenues

Further validation of PCLS is necessary, by directly comparing *in vivo* and *ex vivo* studies, preferably in the same experimental setting, to show the predictive power of PCLS for liver pathology and pharmacological intervention *in vivo*.

Keywords: Liver slices; fibrosis; fibrogenesis; *ex vivo*; ADMET; disease model.
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To improve and accelerate drug discovery, there is an urgent need for reliable and reproducible (animal and especially human) *in vitro* methods to test compounds for the treatment of liver diseases, such as viral hepatitis, NASH, fibrosis, and hepatocellular carcinoma. The *in vitro* models currently utilized in liver research cannot predict or mimic the complex cellular interactions that occur *in vivo*. Thus in isolated primary cells or cell lines, dedifferentiation rapidly occurs, partly due to the loss of the natural environment, including cues from the extracellular matrix and neighbouring or migratory cells. On the other hand, utilization of *in vivo* animal experiments has other shortcomings. *In vivo* studies (1) require large numbers of animals for extended time periods, raising both ethical and financial issues, (2) suffer from interindividual variation, and (3) also have limited implications for human disease, not only due to the notorious lack of appropriate models, but also to relevant species differences in molecular pathogenesis [1]. Therefore, *ex vivo* models are needed that (1) resemble the *in vivo* environment, (2) are reproducible, (3) are low cost and reduce the requirement of live animals, and (4) permit the testing in complex human systems. Precision-cut tissue slices (PCLS) represent an *ex vivo* tissue culture technique that mimics the multicellular characteristics of organs *in vivo*.

Ex vivo liver research started with the pioneering work of Warburg and Krebs in the early twenties [2]. They used liver slices that were prepared manually with limited reproducibility and viability. After a decline in favour of *in vitro* techniques and of another *ex vivo* (liver perfusion) model, the principle was resurrected in the nineties, when the Krumdieck slicer was developed, enabling the production of reproducible and viable tissue slices. In addition, different incubation systems were developed to successfully culture PCLS [3,4], maintaining viability of hepatocytes, Kupffer, endothelial, and hepatic stellate cells [1,5]. Drug transport studies in PCLS clearly showed that besides small molecules, such as digoxin, also larger molecules, like modified human serum albumin, can enter PCLS [6]. De Graaf *et al*. [7] published a detailed description of the preparation and culture of PCLS (see Figure). Another emerging *ex vivo* technique is the decellularized liver extracellular matrix [8]. Compared to the multicellular PCLS that contain a native extracellular matrix (ECM), it uses an altered ECM and is a complex technique. Moreover, to date only single cell types have been used to repopulate these ECM scaffolds.

Current use

PCLS have been used extensively to examine drug metabolism and toxicity. Several studies showed the relevance of PCLS in predicting drug metabolism in the human body [1]. Extrapolations from the results obtained in PCLS to the *in vivo* condition have been successfully established for metabolic clearance, metabolism, and toxicity of several drugs (Table 1). This can be explained by a relatively stable expression of transporters and enzymes that are involved in drug metabolism during culture of PCLS [9]. However, others found decreased expression of certain iso-enzymes and drug transporters (Table 1), which was attributed to the lack of endogenous or exogenous inductive stimuli in PCLS culture medium [9], a problem which needs to be addressed in future studies.

Taking advantage of the multicellular composition of PCLS, different stages of (human) liver fibrosis have been successfully investigated, not only to study mechanisms of fibrogenesis, but also to assess the efficacy of anti-fibrotic agents, using, e.g., the downregulation of procollagen type I gene and protein expression as readout [5,10].

Studies on hepatitis B and C are impeded by their limitation to cell culture and complex *in vivo* disease models. A recent report has demonstrated the feasibility of hepatitis C studies in human PCLS [11]. Furthermore, PCLS are now also used in cancer and metabolic liver disease research [12,13].

Future avenues

Further validation of PCLS is necessary, by directly comparing *in vivo* and *ex vivo* studies, preferably in the same experimental setting, to show the predictive power of PCLS for liver pathology and pharmacological intervention *in vivo*.

Furthermore, PCLS could become an important tool for a personalized medicine. Thus PCLS can be prepared from resected tumor material [12], to determine the most effective cytostatic drug or drug combination *ex vivo*. Precision-cut slices can also be prepared and maintained from other organs [3] enabling serial multi-organ incubations, allowing, e.g., studies on the gut-liver axis [14].

Human PCLS can be cultured for up to 7 days [15], but some functions, such as full metabolic capacity, are maintained only for 3 days (Table 1) [15]. Therefore, PCLS culture conditions should be further optimized to better model the liver *in vivo* over prolonged periods of time. Using exogenous and endogenous inductive stimuli and adding circulating components, e.g., lymphocytes, innate immune cells, chemokines or hormones, could improve the reliability of PCLS. Further progress is also envisaged by perifusing PCLS in a microfluidic system [14]. PCLS are not yet an established tool in drug discovery, likely due to the absence of PCLS cryo-banks that would facilitate material exchange and standardization. Such cryopreservation appears indeed feasible [16].

Conclusion

In summary, PCLS are a unique and promising *ex vivo* system, located in-between experimental and human studies. This technology promises to develop into a pivotal tool to assess hepatic drug metabolism and toxicology, and to test pharmacological agents in various liver diseases.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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