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Review

Kidney-on-a-Chip Technology for Drug-Induced Nephrotoxicity Screening

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Improved model systems to predict drug efficacy, interactions, and drug-induced kidney injury (DIKI) are crucially needed in drug development. Organ-on-a-chip technology is a suitable *in vitro* system because it reproduces the 3D microenvironment. A kidney-on-a-chip can mimic the structural, mechanical, transport, absorptive, and physiological properties of the human kidney. In this review we address the application of state-of-the-art microfluidic culturing techniques, with a focus on culturing kidney proximal tubules, that are promising for the detection of biomarkers that predict drug interactions and DIKI. We also discuss high-throughput screening and the challenges for *in vitro* to *in vivo* extrapolation (IVIVE) that will need to be overcome for successful implementation.

Trends

More physiologically relevant screening devices are needed for drug-induced nephrotoxicity screening because traditional 2D models are poor predictors of *in vivo* biomarkers.

Researchers are increasingly investigating innovative cell types, 3D microfluidic platforms, and biomarker profiling, but biological and technological constraints that challenge their relevance for *in vivo* physiology and biomarker development will need to be overcome.

To become standard in drug screening and biomarker discovery tools, advanced platforms need to be validated against established nephrotoxicity assays and extrapolated to in vivo results.

Prediction of Drug-Induced Kidney Injury

Drug-induced kidney injury (DIKI) is often observed in pharmacotherapy and can be a dose-limiting factor, for example, for the chemotherapeutic cisplatin and the antiviral tenofovir [1,2]. Understanding the (molecular) mechanisms of nephrotoxicity can aid in the development of safer drugs. However, available models do not fully recapitulate the biological functions of the kidney and are poor predictors of DIKI. In many cases, this leads to unexpected effects during clinical studies. Worldwide, enormous efforts are dedicated to develop better predictive models for nephrotoxicity to avoid unnecessary risk for patients and reduce the financial burden of compound attrition in pharmaceutical development. In particular, human *in vitro* models representing relevant kidney functions are needed. Suitable cell systems together with improved culturing technologies will eventually result in bioengineered 3D platforms suitable for nephrotoxicity screening [3], although it has not yet been possible to simulate the complex 3D architecture of a **nephron** (see Glossary) [4]. Implementing physiological parameters such as flow by **organ-on-a-chip** technology has a positive effect on renal cell phenotype, leading to increased sensitivity to toxic agents [5,6]. Clinically relevant DIKI **biomarkers** monitored after drug exposure of kidney-on-a-chip devices need to be established to reflect the *in vivo* situation [7,8].

This review addresses major aspects of a kidney-on-a-chip development that are crucial to improve early prediction of DIKI. In particular, we focus on synergies between state-of-the-art biological and technological innovations.

Cell Biological Requirements of a Kidney-on-a-Chip

The kidney contains more than 10 renal cell types, which are highly organized in a 3D network surrounded by extracellular matrix (ECM) and complex vasculature (Figure 1A,B) [9]. Renal

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excretion of xenobiotics is fulfilled by the concerted action of filtration by glomeruli and (active) secretion and reabsorption by the tubular apparatus. In this review, we focus on processes in the renal tubular system because they are key in the development of DIKI and in **drug interactions** [10] including cellular influx, efflux, and intracellular metabolism, as recently reviewed by Nigam *et al.* (Figure 1C) [11]. Influx can take place at the basolateral and apical membrane of renal **proximal tubule** epithelial cells (PTECs), and is mediated by members of the solute carrier family (SLC). Important transporters in this regard include the organic cation transporter 2 (OCT2/*SLC22A2*) and the organic anion transporters 1 and 3 (OAT1/*SLC22A6*, OAT3/*SLC22A8*) (Figure 1D). Efflux is mediated at the apical membrane via ATP-binding cassette (ABC) transporters, such as P-glycoprotein (Pgp/*ABCB1*), multidrug resistance-associated proteins 2 (MRP2/*ABCC2*), MRP4 (*ABCC4*) and breast cancer resistance protein (BCRP/*ABCG2*). In addition, the multidrug and toxin extrusion transporters 1 (MATE1/*SLC47A1*) and MATE2-K (*SLC47A2*) may play pivotal roles in detoxification through efflux facilitation [12]. Renal drug metabolism is less prominent than in the liver, but is physiologically relevant and involves cytochrome P450 (CYP) and Phase II enzymes [13–16].

Several cellular models are available for drug screening, each with their advantages and disadvantages (Table 1). The majority of current kidney-on-a-chip systems utilize cell lines such as the Madin–Darby canine kidney (MDCK) and the porcine LLC-PK1 (Lilly Laboratories cell, porcine kidney) cells that form tight monolayers and are broadly available, but are of non-human origin. Most of these traditional models have primarily been applied in classical 2D systems. Several well-established human renal models have limited proximal tubular functions, such as the immortalized renal tubular cell line HK-2 (human kidney 2) which has only low-level expression of the SLC22 transporter family [17,18]. It was additionally demonstrated that the HK-2 cell line has undergone many of the early features associated with epithelial-to-mesenchymal transition, based on the pattern of cadherin expression, connexin expression, vectorial active transport, and loss of transepithelial transport [19]. Primary PTECs, freshly isolated from human tissue, are most promising in terms of functionality [7,20,21]. However, this model is hampered by donor-to-donor variability and dedifferentiation upon prolonged cultivation, as well as by possible contamination with other renal cell types owing to the heterogeneity of the kidney [22]. In addition, for screening purposes in pharmaceutical industry, stable cell lines are preferred over primary cells. Hence, for kidney-on-a-chip applications amenable to high-throughput screening, more robust cell models that ensure constant availability and as stable phenotype are needed. Engineered cells, such as the conditionally immortalized human PTEC (ciPTEC) or RPTEC/TERT1 cells, are suitable models for the implementation of bioengineered arrays [23,24]. ciPTECs maintain endogenous expression and functionality of most transporters and metabolic enzymes upon prolonged cultivation in 2D [25,26], making this model promising for applications in 3D systems. Differentiating embryonic stem cells into a PTEC-like phenotype is an alternative promising approach to establish an *in vitro* model, but has not been sufficiently characterized [27]. For a detailed overview of human renal epithelial cell lines, including primary, immortalized and stem cell derived cells, see the review by Tiong *et al.* [9].

Microfluidic Environment Affects Cellular Responses

Traditionally, *in vitro* studies are conducted under static conditions on plastic tissue culture plates or in two-compartment containers separated by semi-permeable membranes. However, PTEC cells *in vivo* are subject to continuous luminal **fluid shear stress** (FSS) and a transepithelial osmotic gradient. *In vivo*, accurate values of FSS on tubules are lacking because urinary flow decreases along the proximal tubule as a result of tubular reabsorption. The flow in the initial portion of the tubule was estimated based on the single-nephron glomerular filtration rate (GFR). In humans, the FSS is estimated to vary between 0.7 dyne/cm^2 and 1.2 dyne/cm^2, but can reach about 1.6 dyne/cm^2 in diseased individuals [28,29]. Studies performed under FSS demonstrated that flow is a key modulator of cellular signal transduction, for which primary cilia are the mechanosensing receptors. Flow affects the organization of cytoskeleton, junctional

Glossary

Biomarker: a biomarker has been defined by Janet Woodcock (FDA) as a 'characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention'. For *in vitro* investigations, biomarkers should ideally be translational (e.g., measurable *in vivo* and *in vitro*) and amenable to be implemented in screening paradigms.

Drug-induced toxicity: the effect by which a pharmacological compound can damage a cell, organ, or organism. The amount of a therapeutic agent causing a toxic effect limits the maximum dose, reduces the safety index of the drug, and may jeopardize patient health.

Drug interaction: the situation in which a compound affects the activity of another compound when administered together. The effect is mainly due to interactions in metabolism or transporter mechanisms.

Etching: the process of cutting into a surface or removal of layers from a surface to create 3D nano/micro-sized designs by chemical treatment. These features are employed directly or are used as negative templates to create microfeatures such as channels and posts by casting with an elastomeric material such as polydimethylsiloxane (PDMS).

Fluid shear stress: the force on a solid boundary caused by a fluid moving along a solid boundary. The dynamic viscosity and velocity of the fluid, as well as the height of the boundary, affect the flow shear stress.

***In vitro* to *in vivo* extrapolation (IVIVE):** predicts the reaction of an organism by transposing data from studies with cells or biological molecules in an artificial environment.

Micromolding: a technique for forming 3D micro/nano structures based on the spontaneous filling of capillary spaces formed between two surfaces in contact, at least one of which has a 'negative' of the features, with a fluid that may be a pre-polymer or suspension of materials that solidify in response to a stimulus such as UV or temperature.

Nephron: functional and structural unit of a kidney.