ImmunoTools *special* Award 2018

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Inflammation, resolution and fibrosis in kidney-on-a-chip

In toxicology, drug development, and kidney research there is an enormous potential for a substantial replacement of animal experiments. The development of strategies to replace whole organs by static *in vitro* models or microfluidic systems has made great progress, but these have not been implemented for routine use due to their inherent complexity, and therefore significant reductions of animal usage remain awaited. Almost all of the available kidney-chip platforms still face critical hurdles such as 3D culture, pump-free flow, and a multiplex 96-well format. Recently, the OrganoPlate® technology invented by Mimetas (Leiden, Netherlands) became available that overcomes all these limitations by combining a pump-free low-flow perfusion system with the possibilities of more complex 3D organ structures in a 40- or 96-well format.

We started to develop and validate different 3D kidney-on-the-chip assays based on the OrganoPlate® format (Fig. 1A) that meet the unique requirements of toxicology, drug development, and experimental kidney research. We are employing primary human renal progenitor cells for seeding and differentiation into mature human renal tubule structures in order to avoid the disadvantages of immortalized cell lines and the uncertainties of induced pluripotent stem cells (iPSC). Such cells can be isolated and cultured from the urine of patients with great potential for expansion *in vitro*. This also offers novel opportunities for personalized medicine, such as functional genomics in rare diseases of the kidney, simulated *in vitro* for pharmacological responsiveness.

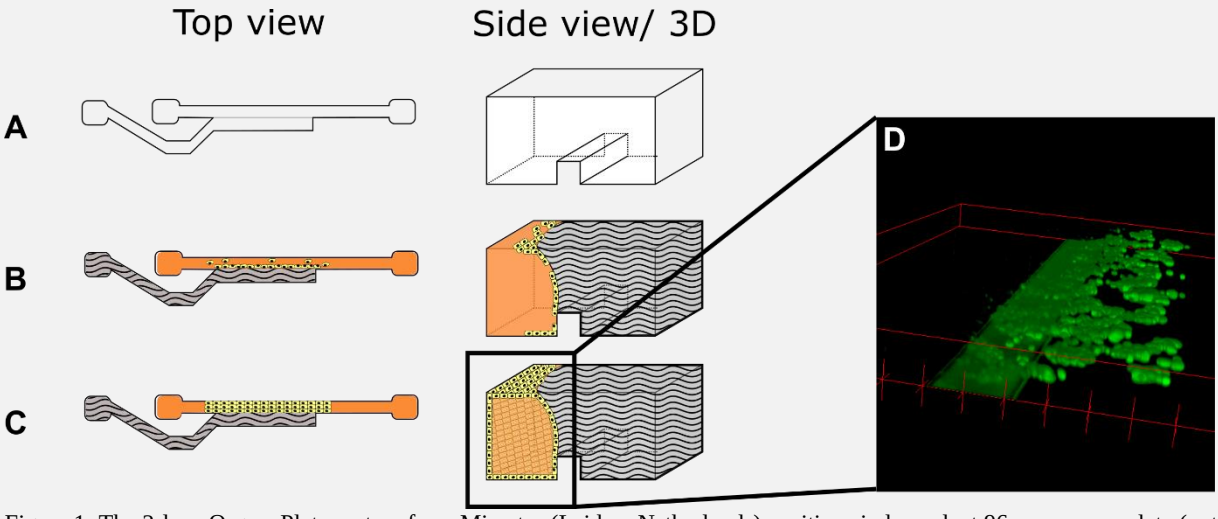

Figure 1: The 2-lane OrganoPlate system from Mimetas (Leiden, Netherlands) positions independent 96 arrays on a plate (not shown), consisting of 3 in- and outlets and 2 separate lanes (A). By loading an extracellular matrix of collagen (B) that forms a meniscus, adherence of cells that mediates formation of 3D tubular structures (C/D) is made possible. Low-flow perfusion is made possible by putting the whole plate on a rocker that enables gravity-driven bi-directional flow of defined speed and force.

Beyond constructing 3D renal tubules for nephrotoxicity screening purposes, assays to mimic acute tubular injury, tubular regeneration and fibrosis after injury could help to reduce animal experiments in kidney research by 50-90%. We already established a protocol to generate and maintain a 3D tubular structure from human-derived renal progenitor cells with an adjacent extracellular matrix (Fig. 1A-D).

This system can be transported from the 2-lane version, that was used for establishing the protocol, shown in Figure 1 to the 3-lane version that contains a third compartment (Fig. 2). This enables us to exploit the co-culture capabilities of this system to its fullest and simultaneously mimicking the *in vivo* conditions of a tubular segment that is surrounded by extracellular matrix and an adjacent supporting vessel (Fig. 2B). In that fashion it will be possible to introduce immune cells, e.g. human primary neutrophils and PBMCs (Fig. 2C) or fibroblasts (Fig. 2D) into the endothelial or matrix compartment, respectively. Now, to further validate and explore our system, we want to simulate different conditions that the kidney parenchyme suffers after an episode of acute injury in the most controlled manner possible. Therefor we will make use of various recombinant cytokines that mimic the micromilieu during acute inflammatory response, resolution of inflammation, parenchymal regeneration or mesenchymal healing (i.e. fibrosis), as listed in Table 1. We will deploy the respective cytokines individually and in appropriate cocktails to understand the contribution of each mediator in the context of the whole micromilieu.

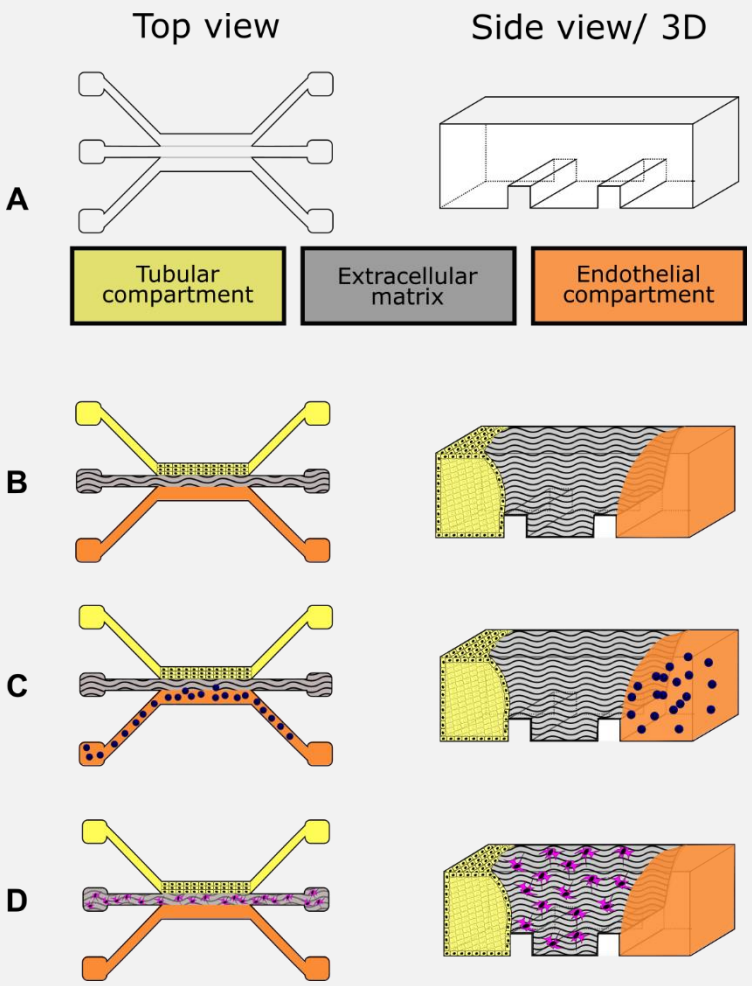

Figure 2: The 3-lane-systems is set up similar to the 2-lane-system, only that there are 2 perfusion compartments on either side of the extracellular matrix channel (A). Hence, the complete 3D tube is connected to a supporting channel (endothelial compartment), only separated by a diffusible barrier of collagen (B). This setup allows for the compartment-specific introduction of further cells, e.g. immune cells in the endothelial lane (C) or myofibroblasts in the matrix lane (D). In a similar manner, cytokine cocktails, that mediate the crosstalk between immune and non-immune cells can be introduced compartment-specific as well.

Phase after injury	**Cytokines**
Early injury phase	rh IL-1beta, rh IL-6, rh IL-8/CXCL8, rh MCP-1 / CCL2, rh GRO-alpha, rh MIP-1b / CCL4, rh TNFα, rh IL-17A, rh IL-17B
Resolution of inflammation	rh IL-1RA, rh IL-4, rh IL-10, rh TGFb1
Regeneration	rh IL-22, rh EGF, rh IGF-I, rh IGF-II, rh BMP-7
Fibrosis	rh IL-4, rh IL-13, rh TGFb, rh FGF-23, rh PDGF-AA, rh PDGF-BB, rh Exodus-2 / CCL21

Table 1: Cytokine cocktail compositions that resemble different phases after tubular injury.

By applying this approach, we will be able to study compartment- and micromilieu-dependent mechanisms during the different phases after tissue injury and thereby validating the functionality of our kidney-on-a-chip approach. Mechanistic analyses include recruitment of PMBCs and neutrophils from the endothelial to the tubular compartment during injury, tubular cell barrier re-establishment during resolution of inflammation and regeneration and proliferation of myofibroblasts as well as their invasion into the injured tubular compartment during fibrogenesis.