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In vitro models in studying nephropharmacology

In vitro systems to study nephropharmacology: 2D *versus* 3D models

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ABSTRACT

The conventional 2-dimensional (2D) cell culture is an invaluable tool in, amongst others, cell biology and experimental pharmacology. However, cells cultured in 2D, on the top of stiff plastic plates lose their phenotypical characteristics and fail in recreating the physiological environment found *in vivo*. This is a fundamental requirement when the goal of the study is to get a rigorous predictive response of human drug action and safety. Recent approaches in the field of renal cell biology are focused on the generation of 3D cell culture models due to the more *bona fide* features that they exhibit and the fact that they are more closely related to the observed physiological conditions, and better predict *in vivo* drug handling. In this review, we describe the currently available 3D *in vitro* models of the kidney, and some future directions for studying renal drug handling, disease modeling and kidney regeneration.

1. Introduction

The kidneys are essential organs in the homeostatic regulation of the human body, able to handle 180 L of plasma filtrate every day to finally excrete about 1.5 L per day in the form of urine containing waste products or foreign substances. This is required to keep our blood and extracellular fluid clean and chemically balanced. The organ further produces hormones (such as renin and erythropoietin), activates Vitamin D, regulates systemic electrolyte balance, pH, and the extracellular fluid. These functions are performed by approximately 1 million units, called nephrons, which can be subdivided into five sections, made up by the glomerulus, the proximal tubule, the loop of Henle, the distal convoluted tubule and the collecting duct. The three main processes that take place in the nephrons are: filtration, reabsorption and secretion (Fig. 1).

Upon entering the nephron capillaries, arterial blood flows through the glomerulus, where filtration occurs under influence of hydrodynamic forces. In a healthy kidney, only substances with a molecular weight up to 7000 Da can freely pass the glomerular filtration barrier. For large molecules, molecular size and charge determine the rate of filtration (Mutsaers et al., 2013). The filtered fluid therefore consists mainly of water and unbound solutes. Once this fluid passes from the glomerulus into the tubular lumen, it becomes part of the body's external environment. To prevent major loss of fluid, almost all of the filtered water is reabsorbed through channels present in the tubular segments of the nephron. Together with water, the proximal tubule cells reabsorb ions such as Na^+ and Ca^{2+}. Na^+ is actively transported into the extracellular fluid by the Na^+-K^+-ATPase localized on the basolateral membrane. By means of facilitated Na^+-coupled transport the proximal tubule is able to reabsorb a wide range of substances such as PO_4^{3-}, amino acids, glucose and organic metabolites (Rosenblatt et al., 2001). Furthermore, tubular transcytosis, endocytosis and pinocytosis can mediate the reabsorption of proteins, hormones and enzymes that have passed through the glomerular filtration barrier.

When the filtrate enters the loop of Henle, urinary concentration takes place (Eisenhoffer et al., 2012). Here a countercurrent exchange facilitates water reabsorption. In the (thick) ascending limb, active reuptake of Na^+, K^+ and Cl^- causes the fluid to become hyposmotic. The distal convoluted tubule then fine-tunes the electrolyte content by facilitating further sodium chloride reabsorption, potassium secretion, and adjusts Ca^{2+} and Mg^{2+}

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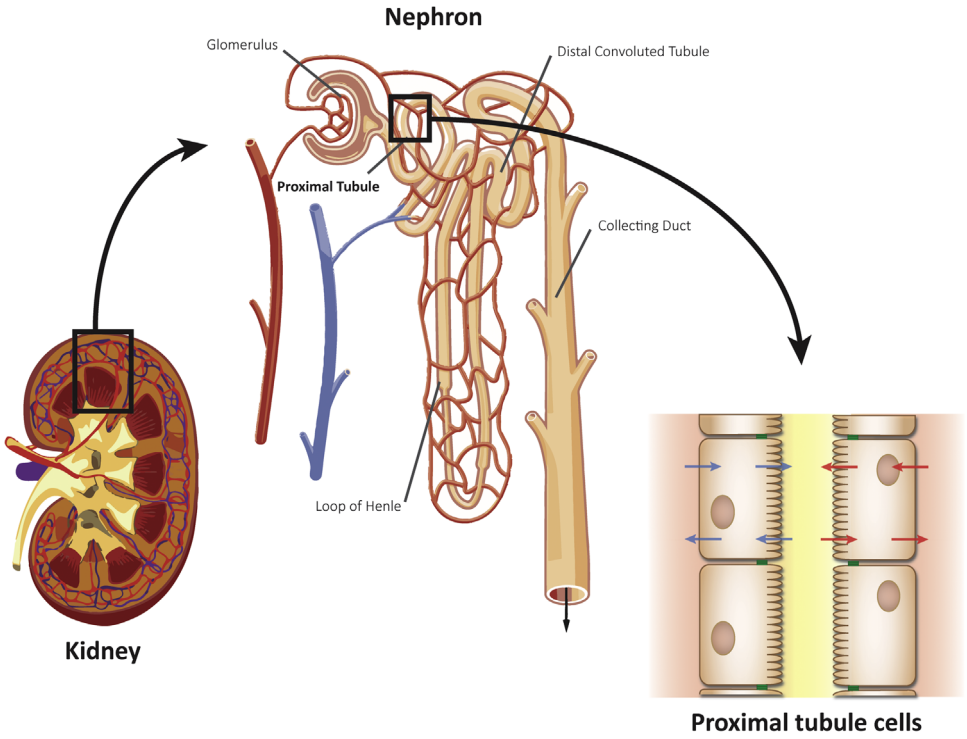

Fig. 1. Kidney and nephron morphology. The human kidney consists of approximately 1 million nephrons and each nephron has a cortical and a medullary portion. The nephron can be subdivided in five sections, made up by the glomerulus, the proximal tubule, the loop of Henle, the distal convoluted tubule and the colleting duct. This review focuses especially on the active solute transport taking place in proximal tubule epithelial cells, as proximal tubular secretion processes play an essential role in the removal of xenobiotics such as environmental chemicals, drugs, or endogenous waste products originating from metabolism.

balance (Blum, 2015). Subsequently, the collecting system, consisting of the connecting tubule and the collecting duct, make the final adjustments in urinary concentration (Eisenhoffer et al., 2012) and it decides the final excretion of potassium and protons, and sodium to some extent.

To enhance urinary excretion of substances, the kidney is able to secrete specific molecules. Secretion is very important for the maintenance of body homeostasis, acid-base balance and the removal of xenobiotics or endogenous solutes. Proximal tubular secretion processes play an essential role in the removal of xenobiotics such as environmental chemicals, drugs, or endogenous waste products originating from metabolism. Due to their high metabolic rates and exposure to toxic agents the proximal tubule cells (PTC) are more exposed to hypoxia and chemical insults than other nephron segments. Accordingly, most *in vitro* models of renal function have focused on reproducing PTC function, which is also the main cell type discussed in this review.

1.1. The proximal tubule cell in renal drug handling

The PTC are well furnished with a variety of transporters with overlapping substrate specificities that cooperate in uptake from the blood (basolateral) compartment and secretion into the pre-urine (luminal compartment). These transporters are often involved in clinically significant interactions, which may lead to unexpected changes in the plasma levels of the compounds involved and/or nephrotoxicity. PTC uptake of organic anions is mediated by members of the solute carrier (*SLC*) family known as organic anion transporter 1 and 3 (OAT1/3; *SLC22A6* and *-A8*) and the bidirectional organic anion transporting peptide 4C1 (OATP4C1; *SLCO4C1*) (Kleinman et al., 1987; Pienta et al., 1991). As the uptake of negatively charged anions is an energy consuming process, the influx transport of OAT1 and 3 is driven by their exchange for intracellular anions, such as dicarboxylates (Chen et al., 2014). The Na^+-dicarboxylate cotransporter (NaDC3; *SLC13A3*), identified in human kidney tissue in 1996, is essential for the maintenance of a cellular dicarboxylate gradient (Handler et al., 1989). The driving force for OATP4C1 has as of yet not been identified. Cellular efflux of organic anions is facilitated by members of the ATP-binding cassette (ABC) transporter family, known as the multidrug resistance proteins 2 and 4 (MRP2/4; *ABCC2* and *-C4*), and breast cancer resistance protein (BCRP; *ABCG2*), through ATP dependent transport (Terryn et al., 2007; Volpe, 2010). Furthermore, the organic anion transporter 4 (OAT4; *SLC22A11*) and the urate reuptake transporter (URAT1; *SLC22A12*) mediate the transport of organic anions by their exchange for urate (Fey-Lamprecht et al., 1998, 2000). Fig. 2 depicts a schematic model of the major organic anion as well as cation transporters in human renal proximal tubular cells.

The uptake of organic cations is mediated by the *SLC22* family of organic cation transporters (OCTs) present at the basolateral membrane of the PTC. At the brush border membrane, the *SLC47* multidrug and toxin extrusion proteins (MATEs) are expressed. OCTs and MATEs transport a wide variety of structurally unrelated organic cations (Lee et al., 2007; Ni et al., 2011; Sato et al., 2005). In the human kidney, OCT2 (*SLC22A2*) is considered one of the most important organic cation influx proteins. Though OCT1 (*SLC22A1*) and OCT3 (*SLC22A3*) are present as well, their renal expression levels are low. In contrast, their transport function in other tissues, such as liver, heart, skeletal muscle, small intestine and lung, is well described (Lee et al., 2009; Oo et al., 2011). In the kidney, the OCT2-mediated basolateral transport of organic cations occurs through facilitated electrogenic diffusion. OCT2 transport proteins make use of the internal negative membrane potential to allow organic cations to enter into the cell. For proper substrate influx, intracellular concentrations need to remain low, as transport direction is determined by the concentration gradient of the substrate. In order to retain those low intracellular levels of cationic