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EDITORIAL

Creating a bioartificial kidney

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Introduction

Worldwide, over 2 million patients suffer from end-stage renal disease (ESRD). The best solution for these patients would be kidney transplantation, however, since transplant options are limited (1), approximately 70% of the patients receive hemodialysis or peritoneal dialysis as replacement therapy. Unfortunately, these therapies have limitations. Both remove low-molecular-weight, water-soluble solutes very well. However, they can only partly remove larger molecular weight solutes and, in fact, they leave protein-bound uremic solutes untouched (2).

In the natural kidney, complete solute removal is achieved by combining the removal of small solutes through glomerular filtration with the removal of the larger ones and protein-bound solutes by the proximal tubule. Actually, the current dialysis therapies only mimic the glomerular function. To achieve a complete treatment these should be combined with a bioartificial kidney (BAK) device mimicking the tubular function. A BAK device can be realized through the creation of 'living membranes' by coupling artificial membranes with functional kidney cells (3), as schematically depicted in Figure 1.

In 1987, the BAK concept was initiated by Aebischer (4). In the following years, devices improved and became more sophisticated, and in 1999 the group of Dr. Humes first utilized porcine renal proximal tubule cells (LLC-PK1) cultured on semipermeable, polysulfone, hollow-fiber membranes. The membranes were coated with pronectin-L to enhance cell attachment and growth (5). The group reported later on the safety and efficacy of BAK use with human primary proximal tubule epithelial cells (hPTEC) in patients with acute renal failure (6). Despite demonstrated essential renal functions, including excretory, metabolic and endocrine pathways and immunomodulatory activities, the phase II trial had to be interrupted due to undesired adverse effects and technical issues.

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In general, 3 major challenges remain to realize BAKs: i) the limited availability of hPTECs capable of transepithelial excretion of uremic retention solutes; ii) the development of a living membrane consisting of tight cellular monolayers maintaining their typical polarity and functionality; and (iii) upscaling of the device for application to patients under good manufacturing practice (GMP) conditions (7). This editorial presents the results obtained at the laboratories of the authors during the last few years towards the first 2 challenges: new cells and the development of living membranes, such as those presented during an invited lecture at the ESAO 2016 conference (Warsaw, Poland, September 2016).

Development of a stable cell source for the bioartificial kidney

Animal renal epithelial cells (porcine [primary or LLC-PK1], monkey [JTC-12] and canine [MDCK]), is not an optimal choice since their application to humans is restricted and their physiology is different from that of renal cells of human origin (8, 9). Some 10 years ago, we started developing human, conditionally immortalized, proximal tubule epithelial cell (ciPTEC) lines (10, 11), because suitable cell lines of human origin were poorly available. We used a urine sample of a healthy human volunteer to isolate proximal tubule cells, which were cultured. The principal limitation of these primary cell cultures, however, was dedifferentiation and the limited number of cell divisions before entering senescence. To overcome the latter problem, the cells were transduced with human telomerase (hTERT), which limits replicative senescence by telomere length maintenance. Furthermore, to control their proliferation, the temperature-sensitive vector SV40tsA58 was introduced, allowing proliferation at 33°C and differentiation into mature PTEC at 37°C. This revealed stable cell lines with intact proximal tubular characteristics and endogenous expression of various functional transport proteins (10). The most prominent uptake transporters with respect to protein-bound uremic solute handling are the organic anion transporter 1 (OAT1/*SLC22A6*), organic anion transporter 3 (OAT3/*SLC22A8*), the organic anion transporter polypeptide (OATP4C1/*SLCO4C1*) and the organic cation transporter 2 (OCT2/*SLC22A2*) belonging to the solute carrier family (SLC) of transporters (see Fig. 2) (12).

The predominant efflux transporters are the ATP-binding cassette (ABC) transporters including breast cancer resistance protein (BCRP/*ABCG2*), P-glycoprotein (P-gp/*ABCB1*), the multidrug resistance proteins 2 and 4 (MRP2/4; *ABCC2/4*) and the multidrug and toxin extrusion 1 and 2 transporters (MATE1 and

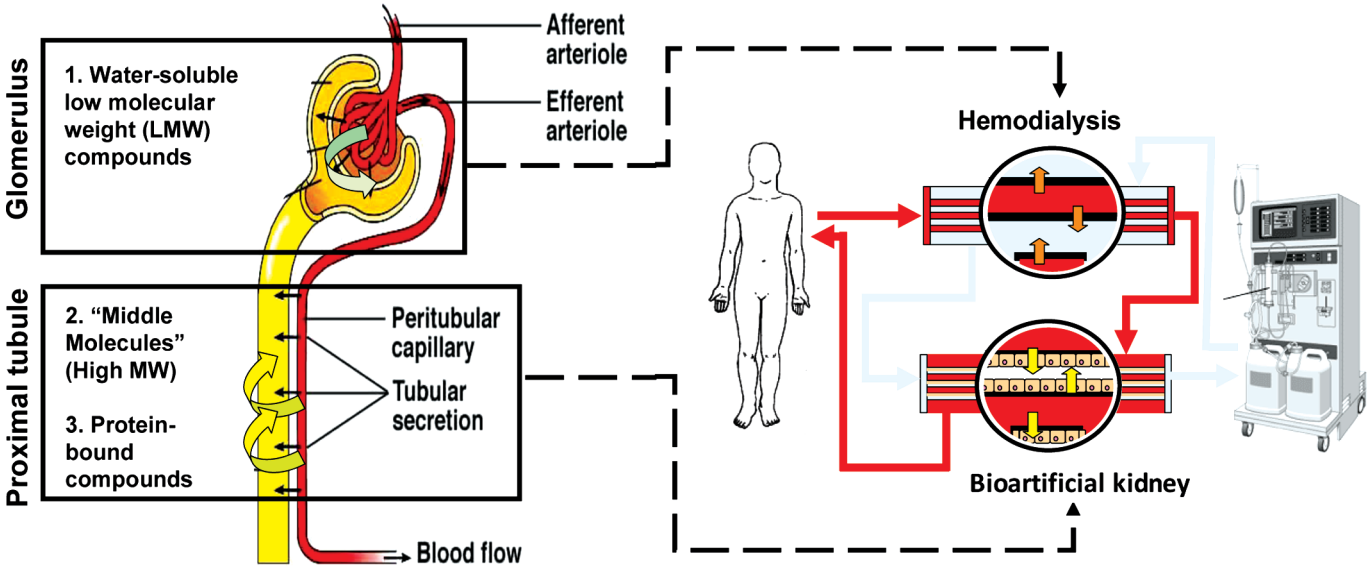

Fig. 1 - Combination of hemodialysis and of the bioartificial kidney for achieving a complete removal of uremic solutes from blood.

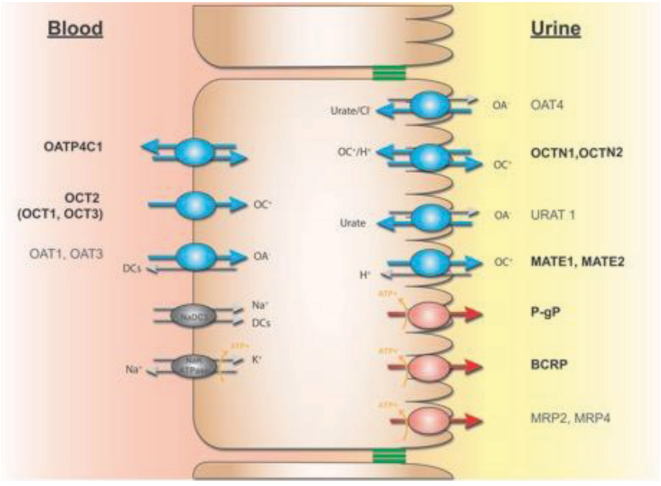

Fig. 2 - Schematic model of the major organic anion (OA-) and organic cation (OC+) transporters in human renal proximal tubular cells. SLC transporters are depicted in **blue** and ABC transporters in **red**. **Grey arrows** depict the movement of driving ions. Transporters that are currently considered important for the clearance of organic cations are labeled in **bold.** Taken from (13) with permission from Elsevier.

2K; *SLC47A1/-2K*) (Fig. 2). As mentioned, PTEC in culture rapidly dedifferentiate and one of the most striking features is the rapid loss of OAT1 and OAT3 within a couple of days. We also experienced this in our ciPTEC and therefore reintroduced the transporters via lentiviral transduction, which revealed highly robust cell lines with regained OAT function that are predictive for tubular handling of renally cleared compounds (14).

Engineering of the "living membrane"

The first step towards the development of the kidney tubule was done by culturing ciPTEC on polyethersulfone (PES)-based flat membranes with a molecular- weight cutoff of 50 kDa (15). To achieve reproducible good quality monolayers, a combination of 2 mg/mL 3,4-dihydroxy-l-phenylalanine (L-DOPA; 4 minutes coating, 1 hour dissolution) and 25 µg/mL collagen IV (Col IV; 4 minutes coating) was applied, which was previously favorable for BAK bioreactors (16). The abundant expression of zonula occludens-1 (ZO-1) indicated a good quality cell monolayer, whereas the permeability marker inulin demonstrated restricted leakage as a result of tight monolayer formation (Fig. 3).

To further evaluate their function, we first set up experiments to validate OCT2-mediated transport, as we demonstrated earlier that cationic uremic solutes interact with that system (17). An endogenous substrate for OCT2 is creatinine (18). We evaluated transport of ^{14}C-creatinine in the monolayers and demonstrated its active secretion (Fig. 3). The addition of the OCT2 inhibitors, metformin or cimetidine, significantly reduced the transepithelial creatinine flux, confirming active OCT2-mediated creatinine uptake in ciPTEC.

In a follow-up study, ciPTECs were successfully cultured on small-sized, MicroPES, hollow-fiber membranes (19). As in the case of the flat membranes, the application of the dual coating (L-DOPA and Collagen IV) was crucial for achieving a homogenous cell monolayer. The abundant expression of the tight junction protein ZO-1 in the cell monolayers proved the polarized and epithelial character of the ciPTEC. The layer also had very low leakage, as demonstrated by the limited inulin-FITC transport through the cell monolayer. Finally, the active OCT2 transport was demonstrated using fluorescent OCT2 substrate 4-(4-(dimethylamino)styryl)-N-methylpyridinium iodide (ASP+) (20). This uptake was inhibited in the presence of a polyamine and guanidino cationic uremic toxin mixture (UTmix) (17), as well as in the presence of cimetidine, a well-known OCT substrate inhibitor (see Fig. 4).

More recently, we successfully developed an upscaled living membrane containing 3 MicroPES, hollow-fiber membranes supporting ciPTECs (21). Abundant expression of ZO-1

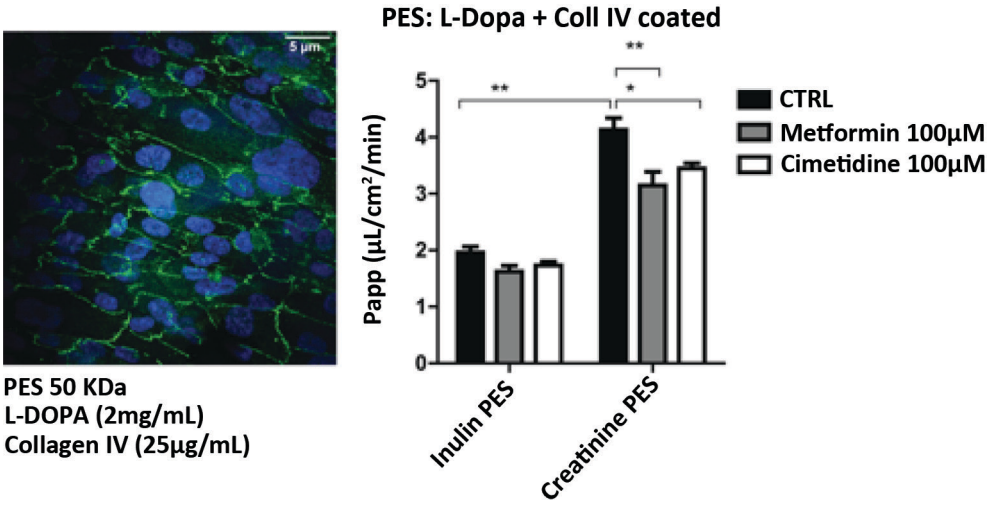

Fig. 3 - Left: Representative images of immunocytochemical analysis of the ZO-1 tight junction protein (**green**) and nuclei (**blue**) in ciPTEC monolayers cultured on PES membrane. Magnification 60×. **Right:** permeability of inulin and creatinine through the cell monolayer. Taken from (15) with permission from Elsevier.

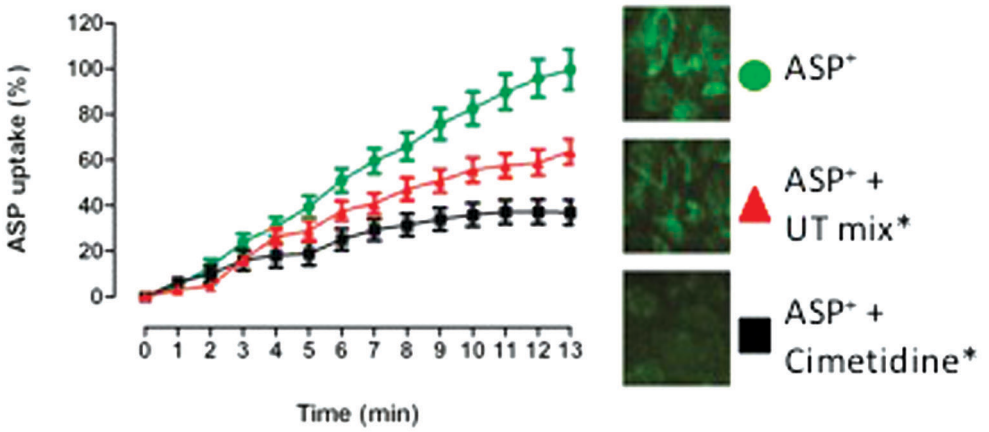

Fig. 4 - Representative real-time images and semi-quantification of ASP^+ uptake by the cells, in the absence (**circles**) or presence of specific inhibitors (**triangles:** cationic uremic toxin mix [UTmix], **square**: cimetidine [100 µM]). Data were normalized against ASP^+ uptake in the absence of inhibitors. Slightly modified from (19), with permission from Nature Publishing Group.

protein along with limited diffusion of FITC-inulin confirmed a clear barrier function of the monolayer. Active ASP^+ uptake by the cells was decreased by 60% in the presence of either UT-mix or cimetidine, proving again the active function of OCT2 (Fig. 5).

In parallel, we also developed bioengineered renal tubules by culturing MicroPES hollow-fiber membranes with ciPTEC-OAT1 (22). Many endogenous metabolites, but also exogenous metabolites (i.e., drugs) are organic anions and need OAT1 for their removal. We demonstrated the secretory clearance of human serum albumin-bound uremic toxins, indoxyl sulfate and kynurenic acid, through a concerted action of OAT1 in uptake and BCRP and MRP4 in their apical efflux. Interestingly, albumin stimulated the transport of indoxyl sulfate and kynurenic acid, which emphasizes the ability of PTEC to shift the protein-binding to the free faction to allow for active secretion. This emphasizes the importance of functionally active kidney cells in renal replacement therapies.

Conclusion - Outlook

An upscaled bioartifiicial kidney tubule with matured ciPTECs representing clear epithelial characteristics with barrier and active transport function was successfully established. In the near future, the upscaled device will be tested for transepithelial clearance of anionic uremic toxins. In addition, culturing the device in a bioreactor system with continuous fluid flow on the cells will be an asset, as this mimics the physiological situation more closely and likely advances the epithelial character of the cell-based system (23). Moreover, testing the safety of the device before its application in humans is essential. Devices with genetically modified cells require strict conditions, tested according to “advanced therapy medicinal products” (ATMPs). A tissue-engineered BAK designed as a combined ATMP, would consist of a medical device, which follows the same basic principles as a dialysis unit, but combined with manipulated cells containing hTERT and SV40T through retroviral transfections. Our cell lines were tested for the presence of endogenous, replication-competent retroviruses and found to be negative and no viral particles formed (data not shown). However, a serious point of concern regarding the use of these vector-infected cells in humans is the increased risk of recombination events due to subsequent virus infections in the patient or activation of endogenous retroviruses. To prevent recombination, additional safety measures, such as co-transduction with a suicidal gene

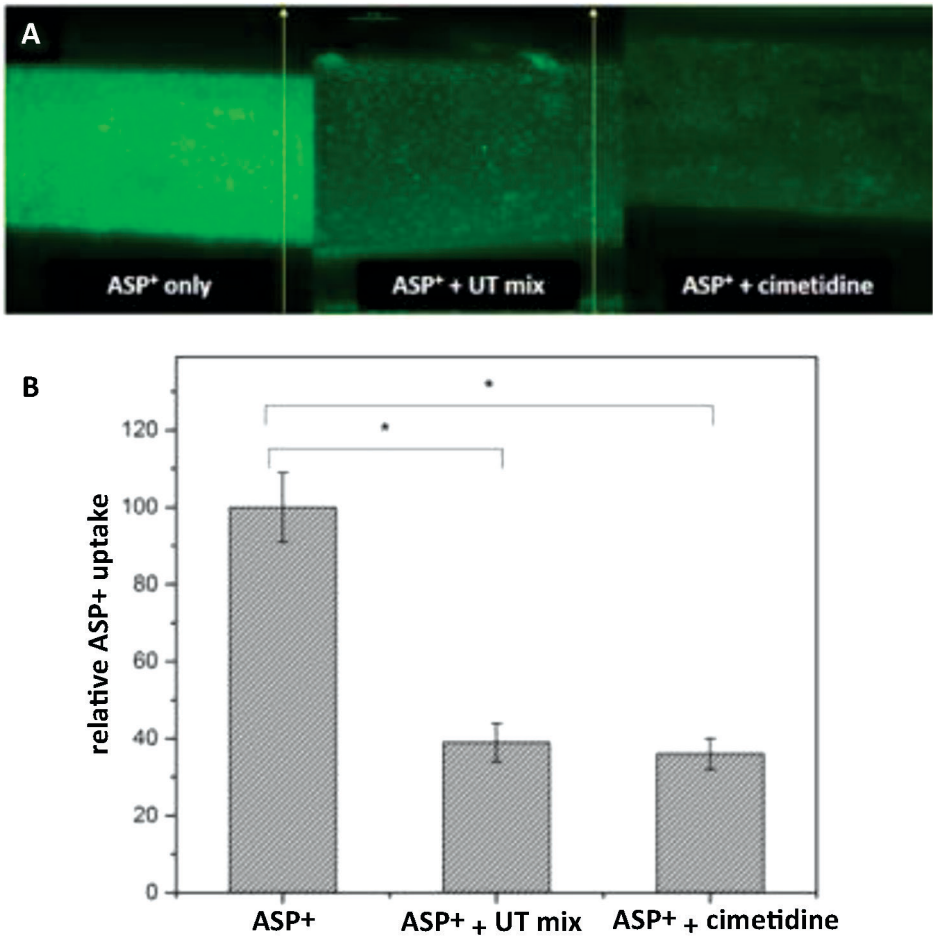

Fig. 5 - (**A**) Representative confocal microscopy images and (**B**) quantification of ASP+ uptake in the absence or presence of cationic uremic toxin mix (UT mix), or cimetidine inhibitor in matured ciPTEC cultured on upscaled bioartificial kidney tubule. Taken from (21) with permission from Elsevier.

or further splitting of the viral genome, might be necessary. Furthermore, the immunogenic effects of applying ciPTECs in a BAK need to be evaluated thoroughly, as the allogeneic cells can exert immune responses. Finally, studies toward the short- and long-term efficiency of uremic solute removal under uremic conditions are indispensable in the preparation toward (pre-)clinical applications.

Disclosures

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