Renal regenerative medicine
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Deterioration of renal function is typically slow but progressive, and therefore renal disease is often diagnosed in a late stage when already serious complaints occur. Ultimately when renal function has dropped below 10%, renal replacement is required. Renal transplantation provides a long-term solution but due to shortage of donor kidneys most patients receive hemodialysis therapy. Although hemodialysis is an effective method to correct disturbances in water and electrolyte balances in the body, it does not substitute for the important endocrine and metabolic renal functions that are critical for homeostasis. Among these functions are, the renal production of renin which controls blood pressure, the secretion of erythropoietin which stimulates the synthesis of red blood cells, and the excretion of protein bound waste products. As a consequence, many dialysis patients remain in poor health. With the development of regenerative medicine, and particularly tissue engineering and novel drug delivery strategies, alternative routes for renal replacement are emerging. Increasing understanding of (stem) cells, growth factors and regeneration in the kidney has contributed to a whole new view on restoration and reconstruction of (parts of) renal tissue that may be used to improve current renal replacement therapies. Here, an overview of critical interactions between cells, growth factors and extracellular matrix molecules in kidney development and regeneration is described. Ultimately, it is discussed how these interactions can be translated to strategies for in-vivo regeneration and in-vitro reconstruction of the kidney. Both strategies are being explored in this thesis.

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1.1 INTRODUCTION

KIDNEY REPLACEMENT THERAPIES

In the Western world end-stage renal disease (ESRD) is becoming an increasing clinical and economical burden.1 Kidney patients need to undergo lifelong renal replacement therapy, which is very expensive and which puts a major burden on the quality of life. Renal transplantation is by far the best renal replacement therapy, but for many kidney patients this is not an option because of the shortage of donor organs, incompatibility problems, and the detrimental effects of long-term use of immunosuppressive drugs. The majority of patients depend on hemodialysis; a therapy that is based on the removal of small molecular waste products and the correction of electrolyte disturbances by passive diffusion over a semi-permeable membrane against a defined dialysis solution. Although the therapy effectively removes small waste molecules and to a lesser extent larger waste molecules, i.e. so-called ‘middle molecules’, medication is still required to control the calcium and phosphate levels and to compensate for the loss of renal production of erythropoietin (Epo).2

Until now, the clearance of uremic waste products, in particular the ‘middle molecules’ with a molecular weight of 500-6000 Da, and the protein-bound uremic toxins, by conventional dialysis therapy is highly inefficient.2 Increasing the pore size and the permeability of the dialysis membranes improve the clearance of ‘middle molecules’ to a certain extent. Another major drawback of hemodialysis is the intermittent nature of the treatment. Nowadays, conventional hemodialysis treatment is performed for approximately four hours thrice a week in specially equipped dialysis clinics. Hence hemodialysis is associated with large swings of the internal milieu that significantly contribute to cardiovascular complications.3 This problem can be partially overcome by peritoneal dialysis. In this therapy the peritoneal membrane is used as a natural semi-permeable filter between the blood and the dialysis solution which is infused in the peritoneal cavity. This technique allows a better and more gradual removal of water and electrolytes, and gives the body more time to adjust. However, the net clearance rate that can be reached with this technique is lower than with hemodialysis. In the long run, fibrosis of the peritoneal membrane is inevitable, and many peritoneal dialysis patients switch to hemodialysis after a few years. In addition, peritoneal dialysis patients are highly susceptible to peritoneal infections.

With respect to dialysis efficiency the limits of the current techniques have almost been reached. Meanwhile, the number of kidney patients increases steadily. It is estimated that at least 8% of the population in the Western world has some evidence of mild renal disease, and are at risk to develop ESRD. The rising costs associated with renal replacement therapy and the growing number of the kidney patients urges the quest for alternative therapies.

TOWARDS NOVEL THERAPIES

With the development of the interdisciplinary field of regenerative medicine4-7, the possibility of restoration of kidney tissue8-12 using (stem) cells, regenerative factors, biomaterials, or combinations of these three, is approaching (Fig. 1). Therapeutic regenerative strategies can be aimed at either enhancing the body’s regenerative capacity or at the reconstruction of new tissues, i.e. tissue engineering. Although regeneration of complete nephron structures in the kidney can only occur in the embryo, repair of damaged renal cells remains possible in adult life provided that the patient is still young and the damage is not too extensive. If that is the case, local delivery of growth factors and/or (stem) cells to enhance tissue reconstruction may be
potentially effective. However, in chronically affected kidneys the damage is irreversible and the loss of tissue architecture makes regeneration virtually impossible.

For this large group of patients, the construction of tissue-engineered kidneys can be considered. This technique aims to reconstruct renal function by using renal epithelial cells that are grown on biomaterial scaffolds that guide proliferation, differentiation and maturation of renal tissue. However this technique is still in its infancy, and faces many hurdles that have not yet been overcome, such as the acquisition of large numbers of cells and the maintenance of viability and function \textit{ex vivo}. Key to success is the reconstruction of the ‘regenerative niche’, i.e. the molecular interactions between growth factors, extracellular matrix materials and cells. However, questions arise such as: How does the regenerative niche look like? Which factors are important and which are redundant? It is now that answers to these questions are beginning to emerge. In many ways, renal regeneration is reminiscent of renal development.\textsuperscript{13-16}

This chapter gives an overview of the field of renal regenerative medicine with respect to engineering concepts using cells, materials and/or regenerative factors. However, basic knowledge about the kidney is essential, which is firstly described. Furthermore, to unravel the ‘renal regenerative niche’, molecular interactions involved in nephrogenesis and renal regeneration have to be taken into account. Finally, the chapter ends with our approach to renal regenerative medicine which is investigated and discussed in this thesis.

**FIGURE 1.** RENAL REGENERATIVE MEDICINE. Three possible strategies, or combinations of them, that can be applied to regenerate and/or engineer renal tissue.

**1.2 KIDNEY ANATOMY AND FUNCTION**

Our kidneys play an essential role in maintaining body homeostasis by excreting excess water, regulating the chemical composition of the blood, removing waste products, and producing important hormones that help to maintain blood pressure, keep healthy bones and prevent anemia.\textsuperscript{2} Specific hormones that are produced by the kidneys are renin, erythropoietin, vitamin D and prostaglandins. The kidney can be roughly divided into three parts: the outer part which is the cortex, the middle section which is the medulla, and the last part, the renal pelvis, in which the urine is collected and drained into the ureter (Fig. 2).
Blood filtration and subsequent reabsorption takes place in the nephron, the smallest functional unit of the kidney (Fig. 2). Each kidney contains approximately 0.5-1 million nephrons, and each nephron consists of a glomerulus, surrounded by a Bowman’s capsule, a proximal tubule, a loop of Henle, and a distal tubule which is connected to a collecting duct. All nephrons drain into the collecting duct system that ends in the renal pelvis. The glomerulus consists of a capillary bed that is covered by visceral epithelial cells, i.e., podocytes. The endothelial cells of the glomerulus and the podocytes are separated by a basement membrane, which is composed of extracellular matrix (ECM) proteins such as collagen IV and laminin, and negatively charged glycoproteins such as heparin sulphate proteoglycans. Plasma constituents up to 36 Å Stokes radius are filtered in the glomerulus. Anionic compounds are difficult to filter because they need to pass the negatively charged filtration barrier of the glomerulus. Every day 180 L of filtrate is generated by the glomerulus. This filtrate contains uremic waste products, but also a variety of molecules that are important for the body such as glucose and amino acids. These important nutrients are reabsorbed in the tubular system. In addition, to prevent excessive water loss 99% of the water that passes the glomerulus is reabsorbed by the renal tubules. Uremic waste products that are not filtered in the glomerulus, either because of size or charge limitations, or because they are bound to plasma albumins are actively secreted in the pre-urine by the proximal tubular epithelial cells.


### 1.3 KIDNEY DEVELOPMENT

**MESENCHYMAL-TO-EPITHELIAL TRANSITION**

The kidneys are formed by a complex interplay between two embryonic structures: the ureteric bud and the metanephric mesenchyme. The ureteric bud is an outgrowth of the mesonephric duct and gives rise to the collecting duct system. The ureteric bud induces the metanephric mesenchyme around its tips to form the Bowman’s capsule, the proximal tubule, the loop of Henle and the distal tubule. This nephron formation requires epithelialization, which occurs by reciprocal signalling between the ureteric bud and the mesenchyme. The trigger for differentiation requires physical contact and is not mediated by diffusible factors. The formation of epithelial cells from the mesenchyme, which is called mesenchymal-to-epithelial transition (MET), is a key event in the development of the nephron. During MET, the cells from the metanephric mesenchyme are induced to lay down ECM components that serve as polarization signals for cytoskeletal organization. The interaction between polarized cells that are attached to
the ECM supports further differentiation into specialized properties of the epithelial apical and baso-lateral membranes.17

THE ROLE OF THE EXTRACELLULAR MATRIX

ECM proteins are early players in the regulation of MET as they determine cell polarization in kidney development.17 Additionally, also in adult renal tissue homeostasis, the ECM plays an important role, which is reflected in the many renal diseases that are associated with aberrant ECM production and remodelling.2,17,20 Roughly two types of ECM can be distinguished: the interstitial ECM which is a loose connective tissue that fills the intercellular spaces, and the basement membrane which is a network of proteins covered by a monolayer of epithelial cells.17,21 The interstitial ECM is predominantly built up of collagen I and III fibers, fibronectin and tenascin. The basement membrane on the other hand primarily contains collagen IV and laminin anchored together by nidogen/entactin and perlecain.22-23 Other (minor) components of the basement membrane include fibronectin, osteonectin, fibulin, agrin, and proteoglycans. Laminin, and in particular its α-chain, has been shown to be involved in the aggregation of the mesenchyme, and in the induction of polarization of the early tubular epithelial cells.24-25 An antibody against laminin-1 blocks MET and subsequent epithelialization of the renal tubule.24 It is very likely that also collagen IV is involved in the aggregation of the mesenchyme, since its appearance coincides with the development of epithelial polarity. In vitro it has been shown that collagen IV is sufficient to induce branching of ureteric buds.26 Fibronectin plays an important role in the formation of glomerular structures. Although the exact composition of the tubular basement membrane in the adult kidney remains elusive, we know that along the tubular segments there is a great heterogeneity in basement membrane constituents which may reflect the functional specificity of the cells of the nephron segments.23 The interplay between the different ECM subtypes is proposed to be critical for development.

The ECM transduces morphogenic signals which aid in polarization and induction of the epithelial phenotype via specific interaction with so-called integrin cell-surface receptors. These integrins are composed of heterodimers of α and β-subunits. To date, at least 18 classes of α, and 8 classes of β-subunits, that form at least 24 heterodimers, are identified.27 These different integrin dimers are proposed to have specific functions in kidney development.28 They play a role in branching morphogenesis and basement membrane organization.29 Furthermore, they show different, but also overlapping, ligand-binding properties. Integrins specifically interact with peptide sequences derived from different ECM proteins. The RGD (Arg-Gly-Asp)30-31 sequence is the most well-known integrin binding peptide, and it is conserved between collagen, vitronectin, laminin and fibronectin. Other examples of integrin binding peptide sequences are the PHSRN (Pro-His-Ser-Arg-Asn)32-33 derived from fibronectin, DGEA (Asp-Gly-Glu-Ala)34 present in collagen, and IKVAV (Ile-Lys-Val-Ala-Val)35-36 derived from laminin. The α-subunit contains several sites with homology to the Ca2+-binding domain of calmodulin; ligand binding requires Mg2+ or Ca2+. Integrins are linked to the actin microfilaments of the cytoskeleton via cytoplasmic proteins such as vinculin, ezrin, and talin.37 Ligand binding triggers a complex modulation of cell behaviour, such as proliferation, migration, cell survival or apoptosis, and differentiation. Integrin signalling can modulate responses to growth factors and interfere in developmental pathways such as the Wnt-pathway.
IMPORTANT OF INTERCELLULAR ADHESION MOLECULES

Although initially the polarization of renal epithelial cells is specified by the ECM, the interaction with neighbouring cells is important for further specification of the epithelium in later stages of development. Most interactions between cells in the kidneys are mediated via cadherins, which are Ca\(^{2+}\)-dependent adhesion molecules. Epithelial cadherin (E-cadherin) is exclusively expressed on epithelial cells forming adherens junctions by intercellular, homotypic interaction with neighbouring cells. In addition, they can also form intracellular homodimers, and interact with α, β, and γ-catenin, i.e. plakoglobin. In this way, E-cadherins intracellularly activate signal transduction pathways such as the Wnt/β-catenin pathway. Furthermore, they are able to interact with receptor tyrosine kinases (RTK), and in that way alter both cell-cell and RTK signalling. Via mechanotransduction by coupling to the actin cytoskeleton E-cadherins are able to regulate cell shape changes, polarity and morphogenesis. Besides E-cadherin, also epithelial cell adhesion molecule (EpCAM) is involved in nephrogenesis in which its spatio-temporal expression pattern changes in time. Also in the adult kidney the expression of EpCAM varies along the nephron.

PARACRINE SIGNALLING

In the developing kidney, morphogenic stimuli are mainly delivered in a paracrine or autocrine fashion by the following growth factors: transforming growth factors α and β (TGFα, TGFβ), bone morphogenetic protein 7 (BMP7), epidermal growth factor (EGF), hepatocyte growth factor (HGF), insulin-like growth factor (IGF), platelet derived growth factor (PDGF), and fibroblast growth factor (FGF). TGFβ1 is growth inhibitory and triggers epithelial-to-mesenchymal transition (EMT) in renal epithelial cells. It is expressed in the nephrogenic mesenchyme, and induces ECM production by mesenchymal cells. Moreover, TGFβ1 contributes to the deposition and maintenance of ECM in the kidney. In contrast, BMP7 counteracts TGFβ. Activation of the EGF-receptor (EGFR) triggers a strong proliferative response in renal epithelial cells. Although EGF is often used as a mitogen for in-vitro cell cultures, it is hardly detected in developing kidneys. On the contrary, TGFα is found to be the dominant EGFR ligand in nephrogenesis. In adult kidneys, EGF is expressed but its soluble level rapidly drops after damage, probably because of the loss of EGF-producing cells. Instead, a rapid increase is observed of heparin-bound EGF which drives the division of remaining cells. HGF is involved in branching morphogenesis of the renal tubular system. mRNAs encoding IGF genes are found in developing kidneys. Additionally, IGF I and II display a mitogenic effect in cultured renal epithelial cells. PDGF is mitogenic for mesangial cells, and glomerular endothelial cells proliferate in response to PDGF. This suggests an important role in the development and maintenance of glomerular structures. Studies in embryonic kidneys have suggested a role for FGF in renal angiogenesis.

CONCLUSION

The development of the kidney results from a complex interplay between ureteric bud and metanephric mesenchyme. A key event in renal development is the mesenchymal-to-epithelial transition, which is orchestrated by the ECM, cell adhesion molecules and growth factors that are expressed in a spatio-temporal fashion.
1.4 KIDNEY REGENERATION

THE QUEST FOR RESIDENT RENAL STEM/PROGENITOR CELLS

During kidney regeneration, the renal embryonic program is partially recapitulated. However, no complete nephron structures can be formed in the regeneration process. Nevertheless, during adult life the renal tissue is maintained by slow turnover of cells. Only in response to injury, the number of proliferating cells in the kidney, in particular in the proximal tubular part of the nephron, rapidly increases. The increase in proliferation is associated with the re-expression of developmental genes such as Pax2 and Wnt4. To date, many investigators have searched for small populations of resident stem cells that might be responsible for the rapid proliferative response after tissue damage. In healthy kidneys, endogenous renal stem cells would remain in a dormant state, and only start proliferating after damage. In first attempts to identify renal stem cells investigators have applied BrdU label incorporation methods. In rat kidneys label-retaining cells were discovered among tubular epithelial cells which could proliferate in response to injury. Using similar methods a putative renal stem cell population was detected in the papilla. In addition, putative endogenous renal stem cells were also isolated on the basis of the expression of common stem cell markers such as Sca-1, CD133 and CD24. Interestingly, there is some evidence that these cells in response to acute injury can directly incorporate into renal tubules after infusion. Moreover, multipotent renal progenitor cells were isolated from rat kidneys, and have been shown to be able to differentiate into renal tubules when injected under the renal capsule. However, the significance of these observations for renal repair is unclear.

EPITHELIAL-TO-MESENCHYMAL TRANSITION

Genetic fate mapping was used to provide definitive proof that proliferation of surviving tubular epithelial cells is the predominant mechanism of renal repair. Our current understanding is that resident epithelial cells can undergo a regulated process of dedifferentiation, proliferation and redifferentiation. Typically, dedifferentiation proceeds in roughly the reverse order of the process of epithelial polarization. Renal tubular epithelial cells are able to gain a mesenchymal phenotype in response to the loss of basement membrane contact, or in response to several growth factors. This process is called epithelial-to-mesenchymal transition (EMT) and is characterized by the loss of E-cadherin gene expression and the gain of expression of mesenchymal markers such as vimentin, α-smooth muscle actin and SM22α. Moreover, during EMT the basement membrane is destructed and the dedifferentiated epithelial cell, which now has become a myofibroblast, has gained the capacity to migrate. Myofibroblasts can produce large amounts of interstitial collagens, such as collagen type I and III, which may lead to fibrosis. In the kidney EMT is induced by several local factors such as, TGFβ, interleukin 1 (IL1), matrix metalloproteinase 2 (MMP2), FGF2, EGF, advanced glycation end products (AGEs), angiotensin II or reactive oxygen species. Administration of HGF, BMP7, Epo have been shown to block EMT, and prevent fibrosis. Whether EMT truly occurs in vivo or only in vitro is currently under debate.

THE CONTRIBUTION OF BONE MARROW DERIVED CELLS

In addition, bone marrow derived cells (BMDC) may also contribute to renal repair. A number of studies have shown that BMDC are able to contribute to the repair of glomerular endothelial cells, mesangial cells and podocytes in the kidney. The participation of BMDC in
tubular repair was confirmed in human studies. Nevertheless, their contribution to tubular regeneration is very low; only a very low percentage of around approximately 1-2% of the dividing tubular cells after injury originate from the bone marrow. Nevertheless, infusion of BMDC after acute kidney injury prevented the decline in glomerular filtration, suggesting that BMDC may have a paracrine protective role after injury. However, in the case of kidney fibrosis, infusion of bone marrow cells has shown to be ineffective. There is even evidence that BMDC may differentiate towards myofibroblasts, and produce excessive collagens, as described below in more detail.

**CONCLUSION**

Renal epithelial cells are able to undergo a regulated process of dedifferentiation, proliferation and redifferentiation, i.e. EMT and MET. The presence of an intact microenvironment is important for regeneration because without the appropriate signals renal cells cannot exert their functions. In chronic and end-stage renal diseases, the affected kidney is in most cases ‘beyond repair’.

**1.5 EMERGING CONCEPTS IN RENAL REGENERATION AND TISSUE ENGINEERING**

**NOVEL THERAPIES**

Alternative therapies are proposed to be developed via two strategies: i. development of early detection methods and smart renal regeneration therapies, in order to intervene in and cure kidney diseases at an early stage, and ii. design and development of alternative renal replacement therapies, such as a bioartificial kidney (devices) using tissue engineering strategies. As discussed, the possibility of restoration of kidney tissue using cells, regenerative factors, biomaterials, or combinations of these three, is approaching (Fig. 1). Cell-based and factor-based approaches might be able to intervene in impaired kidney functions by induction of renal regeneration. Cell-based approach might also be applied to grow neo-kidneys. Material-based strategies might be used to design and develop a bioartificial kidney (device).

**CELL-BASED APPROACHES: STEM/PROGENITOR CELL INJECTION**

A large number of studies have reported on the contribution of BMDC to renal regeneration. These cells might be injected in the kidney or in the blood to ultimately end up in the impaired kidney. Several studies have shown that BMDC after damage can replace tubular cells, mesangial cells, podocytes, interstitial cells and endothelial cells. But except for interstitial engraftment, the relative contribution is very low and does not exceed a few percent of the total proliferating cell fraction. There is large body of evidence for a paracrine role of BMDC in the repair process. BMDC are recruited after damage, and their incorporation rate is enhanced. Moreover, they produce a variety of paracrine factors, such as VEGF, IGF, bFGF, HGF, TGFβ that can enhance repair. Despite the optimism about the use of BMDC for renal regeneration, there is ample reason to be cautious. It has been shown that almost 30% of myofibroblasts in fibrotic kidneys are bone marrow-derived. Tight regulation of BMDC differentiation in the interstitium is therefore important. It seems that BMDC are able to give rise to multiple cell types depending on the local composition of the environment, i.e. the ECM and growth factors present. For this reason, one should be cautious using BMDC for local
regeneration therapy. An alternative strategy would be to use BMDC that have been differentiated \textit{ex vivo} towards a specific renal lineage prior to implantation.\textsuperscript{85-86} Furthermore, it is important to notice that the uremic environment of ESRD patients functionally impairs progenitor cells such as endothelial progenitor cells.\textsuperscript{87-90}

**CELL-BASED APPROACHES: NEO-KIDNEYS**

Another interesting approach is the formation of neo-kidneys from implanted embryonic tissue, i.e. renal primordial.\textsuperscript{26,91} A great advantage of this approach is that during maturation the tissue is vascularized by the host, which improves compatibility. However, a major obstacle is the source of embryonic tissue, which is closely connected to ethical issues. In future, these cells might be derived from embryonic stem cells (ESC) that are induced towards the renal epithelial lineage. In teratomas from undifferentiated murine ESC, mesonephric and ureteric bud-like structures have been observed, suggesting that differentiation towards renal lineage is possible.\textsuperscript{92} A number of studies have explored the possibility to generate renal epithelia from ESC.\textsuperscript{92-98} After injection, murine ESC have been shown to integrate into the tubuli of developing kidneys.\textsuperscript{94,96,98} Differentiation towards a specific lineage is very complicated and depends on factors secreted in the environment of the developing kidney; this is difficult to mimic \textit{in vitro}. Nevertheless, studies have shown that ESC, after formation of embryoid bodies, were able to differentiate towards the renal epithelial lineage by administration of either BMP4, or by a combination of activin A, BMP7 and retinoic acid, or by gene transfection with the Wnt4 gene.\textsuperscript{93-94,96} Also, the materials and ECM components on which ESC grow \textit{in vitro} are important factors in renal tubule formation.\textsuperscript{99-100} Although this approach seems very attractive for renal regeneration and/or replacement, the use of embryonic tissues is closely connected to ethical and safety issues. However, an exciting study showed that both renal glomerular and tubular structures could originate from single primary renal cells cultured in a three-dimensional environment.\textsuperscript{101} Furthermore, other studies have shown that neo-kidneys could be produced using decellularized kidneys as scaffold.\textsuperscript{102-103} It is clear from these studies that immense progress is made using cell-based approaches. However the step from \textit{in-vitro} to real \textit{in-vivo} kidney regeneration using different renal cells or stem/progenitor cells has a long way to go.

**FACTOR-BASED APPROACHES: DELIVERY OF KIDNEY REGENERATING FACTORS**

Exogenous growth factor administration to enhance renal regeneration has been studied in large detail.\textsuperscript{104} A number of studies, in which up-regulation of growth factor genes in relation to renal repair processes has been found, have provided the rationale for growth factor delivery strategies. Systemic injection of EGF and HGF successfully enhanced recovery and survival after acute kidney injury in animal models.\textsuperscript{104} In addition, systemic administration of IGF1 was evaluated in human trials, and has shown to improve recovery after acute kidney injury.\textsuperscript{105} Furthermore, many examples have been shown in which BMP7\textsuperscript{106} was delivered intravenously\textsuperscript{107} or intraperitoneally\textsuperscript{108}, and resulted in recovery of renal function in animal models.\textsuperscript{109} However, a major disadvantage is that these therapies might lead to unwanted side effects in other organs, especially when the drug is administered systemically. These growth factors show pleiotropic effects, which implies that they need to be targeted to a specific site in the body. Besides that, the stability of these proteins is generally very low; therefore growth factors need to be administered repeatedly. For example, BMP7 has a serum half life of 30 min, and can be found in the kidney shortly after intravenous administration.\textsuperscript{107} Good control over the exact growth factor concentration is therefore important. For example, a low dose of BMP7...
has shown to stimulate epithelial cell proliferation, whereas a high dose has shown to inhibit proliferation and induce apoptosis in the regenerating epithelial tubule. The recovery of renal function by systemic administration of one growth factor is not very likely to reverse or alter the progression of renal disease. Correct regeneration requires the delivery of multiple growth factors in a spatiotemporally controlled way. Therefore sophisticated local delivery and/or targeting systems need to be developed to successfully enhance renal repair.

MATERIAL-BASED APPROACHES: BIOARTIFICIAL KIDNEYS

In the future, a possible solution for patients with ESRD, besides dialysis or transplantation, might be a tissue-engineered kidney. Ideally, a tissue-engineered kidney should be able to replace all kidney functions, including important endocrine and metabolic activities, such as vitamin D3-activation, Epo production, and removal of uremic protein-bound waste products. In view of the extremely complex renal architecture and the great variety of cell types, i.e. more than 15 different cells, bioengineering of a complete neo-kidney is virtually impossible. Therefore, another approach is the bioengineering of an extracorporeal renal device using a membrane and a single renal cell type that has to form a monolayer of cells in order to ultimately replace critical endocrine and metabolic renal functions. Such a bioartificial kidney might be applied as a renal assist device (RAD), and exert its function when placed in series with a conventional hemodialysis module. Many researchers have used renal epithelial cell lines from porcine or canine origin, i.e. respectively Lewis lung cancer-porcine kidney 1 (LLC-PK1) cells and Madin-Darby canine kidney (MDCK) cells. Although, confluent layers of MDCK cells on polycarbonate membranes initially displayed active Na⁺ transport, functional properties could not be maintained after 2 weeks. Moreover, the loss of function was associated with the aberrant distribution of Na⁺/K⁺ ATPase, multilayer growth, and necrosis. Reabsorption of water, glucose and sodium could be maintained up to 10 days when LLC-PK1 cells were used. Importantly, the type of membrane material and the ECM coating appeared to be critical for the adhesion and functional differentiation of renal epithelial cells.

Humes et al applied human renal epithelial cells in a hollow fiber cartridge to replace endocrine and metabolic renal functions in uremic dogs. During 24 h the epithelial cells in the cartridge displayed ammonia excretion, and 1,25-dihydroxyvitamin D3 conversion. Unfortunately, transport of electrolytes was virtually absent. This was most likely caused by the incomplete cell coverage of the cartridge membranes. Despite the limited transport function, the activity of the renal epithelial cells could attenuate the consequences of septic shock by modulating plasma cytokine levels. To this end, initial phase I and II clinical trials have indicated that RAD treatment for 72 h improved long-term survival and renal recovery of patients with acute renal failure.

The application of renal epithelial cells ex vivo is limited by the rapid loss of renal epithelial cell characteristics. Exposure of renal epithelial cells to an atypical physicochemical environment, e.g. fluctuations of glucose levels, lactate accumulation, hypoxia or hyperoxia, an aberrant ECM, and lack of heterotypic cell interactions, contribute to dedifferentiation events. Renal epithelial cell cultures under organotypic perfusion culture conditions, as proposed by Minuth et al might help to maintain differentiated renal epithelial cell features.

The vast majority of studies in this field have been focused on reconstruction of the renal tubular system. However, a bioartificial tubular system is only feasible if it is also connected to a hemofiltration device. Therefore, the RAD is connected to a hemodialysis module. Furthermore,
development of a hemofiltration device which can be implanted in vivo is an extreme challenge; especially because almost every implantable material will elicit a foreign body response, which eventually might result in calcification and encapsulation of the hemofilter. An implantable hemofilter has been created by implanting synthetic hollow fiber membranes around the femoral vascular pedicle in rats. After implantation, the membrane was surrounded by neocapillaries that generated transudate which accumulated in the hollow fiber membrane from which the fluid was externalized. Although the implant generated fluid and permselectivity was observed, the volume that could be generated by this implant remained small. Nevertheless, important progress was made through the development of a renal assist device, and an implantable hemofilter. It is a great challenge to improve these systems in such a way that the epithelial cells within the device encounter a regenerative, natural renal niche.

1.6 OUR APPROACH TO RENAL REGENERATIVE MEDICINE

Alternative therapies for hemodialysis and renal transplantation using different approaches based on renal regeneration using (stem) cells or regenerative factors, or renal bioengineering applying biomaterials, were discussed. Owing to its complex structure, its composition with approximately 1 million nephrons consisting of more than 15 cell types with different functions, it is difficult to specifically influence and/or engineer a (part of) the kidney. Using regenerative medicine the complex interplay between the different renal cells, growth factors and ECM molecules might be influenced and/or engineered. However, it is important to do this stepwise.

AIM OF THE THESIS

Inspired by the complex kidney structure, and the interesting interplay between cells, growth factors and ECM, we propose two strategies to renal regenerative medicine: one at improvement of the dialysis therapy, and one at regeneration of the kidney after acute kidney injury. Our first, in-vitro approach aims at bioengineering of living renal membranes which might be applied in a bioartificial kidney device coupled in series with a dialysis module (Fig. 3A). Whereas in our second, in-vivo approach we aim at renal drug delivery from a carrier implanted under the renal capsule (Fig. 3B). This approach might in future be employed for transplantation kidneys.

In this thesis, we describe the use of supramolecular biomaterials based on four-fold hydrogen bonding ureido-pyrimidinone (UPy) moieties for the creation of synthetic membranes to be applied in a bioartificial kidney set-up, and for the creation of hydrogel carriers as drug delivery vehicles (Fig. 3 and 4). We propose that they have similar dynamics, hierarchical assembly behaviour and nonlinearity as the cells and tissues they will encounter. Furthermore, these materials can be easily made bioactive by using several bioactive modules with the same hydrogen bonding UPy-unit.

OUTLINE OF THE THESIS

The feasibility of our renal regenerative medicine ideas as well as the use of supramolecular biomaterials for this purpose is described in this thesis. First, an overview of supramolecular four-fold hydrogen bonding supramolecular polymers is given, and the state of the art of supramolecular biomaterials is addressed in chapter 2. Furthermore, the importance of
supramolecular chemistry and biomaterials is shown by discussing factors as mechanics, bioactivity, hierarchical assembly, nonlinearity, and dynamics.

Our first renal regenerative medicine strategy is shown in chapter 3 in which hierarchical supramolecular membranes are investigated on their capability to induce renal epithelial cells to form monolayers and become polarized in a perfusion set-up. In chapter 4 we describe the investigation of these membranes in more detail, and bioactive ECM-derived peptide sequences are introduced into these membranes. In this way both the hierarchical fiber-like morphology and the display of bioactive signals of the ECM is mimicked. It is investigated whether renal epithelial cells are able to gain an epithelial phenotype on these bioactive membranes when cultured in a gradient perfusion system. In this way living renal membranes are produced that consist of synthetic basement membranes and renal epithelial cells derived from nephrectomised kidneys.

We ultimately prefer to obtain living membranes in which epithelial cells form a monolayer at one side and endothelial cells at the other side of the membrane (Fig. 3A). For that purpose we investigated two possible endothelial cell sources: endothelial progenitor cells from the blood of chronic kidney patients, and primary endothelial cells derived from nephrectomised kidneys, as described in chapter 5. Both endothelial cell types are seeded on our supramolecular biomaterials to investigate differences in cell behaviour of these cells on these materials.

**FIGURE 3. OUR APPROACH TO RENAL REGENERATIVE MEDICINE.** Two approaches are investigated, A. the bioengineering of living renal membranes, and B. renal drug delivery.

Our second strategy, the renal drug delivery approach to improve impaired kidney function by induction of renal regeneration is shown in chapter 6. First, the foreign body response in vivo to different supramolecular hydrogel carriers is studied after subcapsular implantation in healthy rat kidneys. Then in chapter 7, an ideal supramolecular hydrogel carrier is chosen to investigate the delivery of the anti-fibrotic and anti-inflammatory growth factor protein BMP7 when implanted under the kidney capsule in healthy rat kidneys. In chapter 8, an ischemia-reperfusion injury model in rats is used to prove the renal drug delivery strategy in injured kidneys using the BMP7 containing hydrogel carrier which was selected in chapter 7. Finally in our two strategies and possible versatile scaffolds will be surveyed with respect to their applicability in renal regenerative medicine.
FIGURE 4. SUPRAMOLECULAR BIOMATERIALS. Versatile four-fold hydrogen bonding ureido-pyrimidinone (UPy) based materials will be used as membranes or as drug carriers. In these materials, UPy-UPy dimers are formed that assemble in long nano-fibrillar stacks via lateral urea hydrogen bonding.

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