Development of novel strategies to regenerate the human kidney

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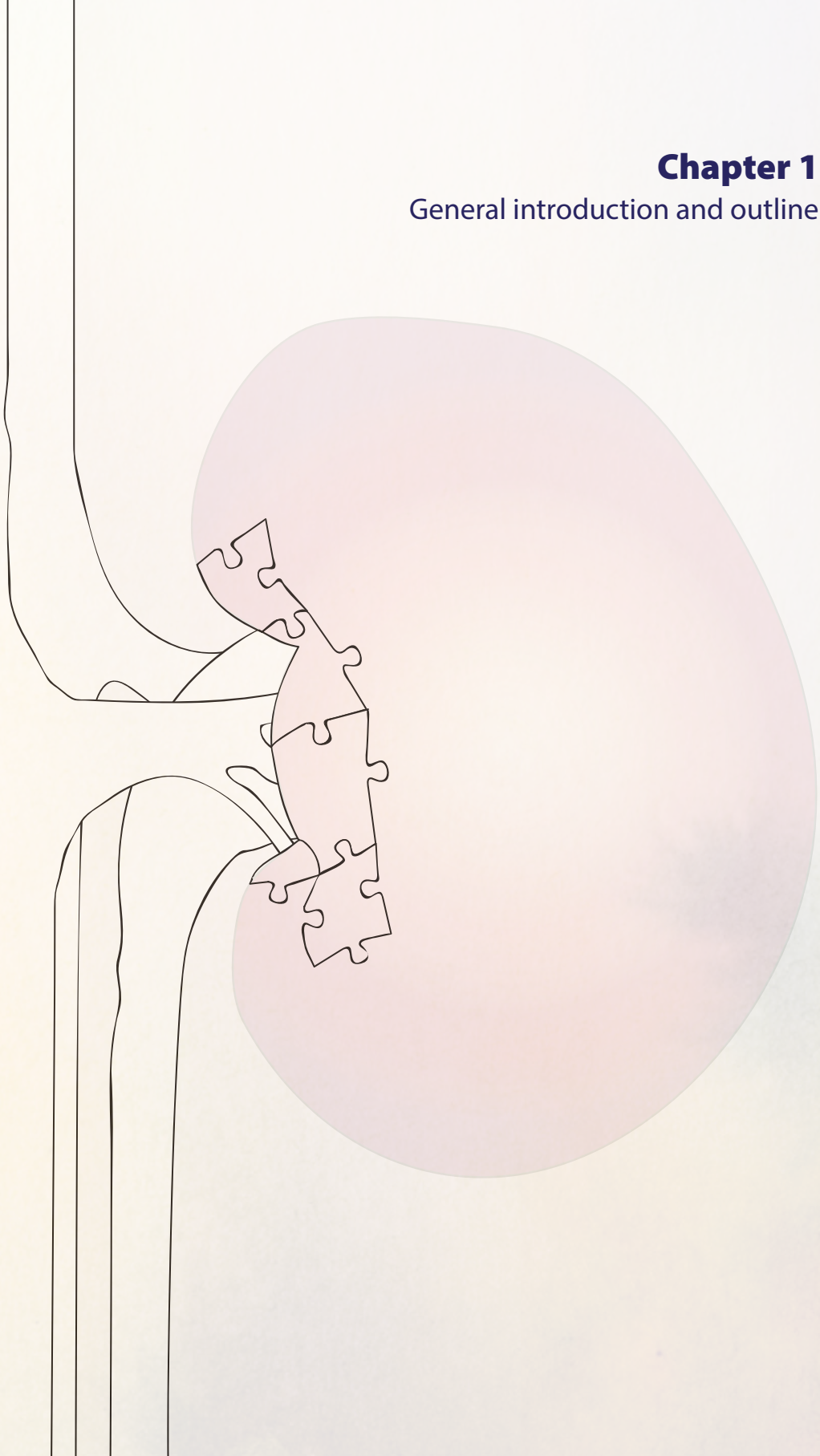
Chapter 1

General introduction and outline



Introduction

Chronic kidney disease (CKD) is a common disease in the western population as at least 8% of this population has a degree of CKD, placing them at a moderate to high risk to develop kidney failure[1]. This figure is increasing each year due to an aging population and an increase in the prevalence of diabetes and chronic vascular morbidity. Therefore, if the present trend continues, the number of people with CKD will double over the next decade[2].

Although there are strategies to slow down the progression to end stage renal disease (ESRD), there are currently no therapies to cure CKD. Therefore approximately 5% of CKD patients will progress into ESRD with the need of renal replacement therapy (RRT)[3]. Currently there are two RRT options for patients with ESRD: dialysis and kidney transplantation. Due to the lower mortality and higher quality of life, the best therapy at the moment is kidney transplantation[2].

However, as there is a shortage of organ donors, patients awaiting transplantation often need dialysis for several years. Moreover, long term outcomes after kidney transplantation are compromised by the effects of rejection and nephrotoxicity of immunosuppressive therapies[2,4]. There is therefore a need for novel treatment strategies to either prolong the survival of transplanted organs or to increase the availability of (bio-engineered) transplantable kidneys.

Mesenchymal stromal cell therapy

One strategy to improve graft survival is the enhancement of the immunological acceptance of the graft, preventing fibrosis and fostering the regenerative capacity of the transplanted kidney. Mesenchymal stromal cell (MSC) based therapy shows to be a promising candidate for these aspects as their properties may influence both inflammation and fibrosis[2].

Mesenchymal stromal cells are perivascular located cells originally isolated from the bone marrow (bmMSCs). Due to their perivascular location, MSCs can closely interact with several cell types including endothelial cells, resident macrophages, dendritic cells (DCs) and recruited inflammatory cells (Figure 1)[2]. Due to these interactions, MSCs show strong tissue homeostatic and immunomodulatory properties.

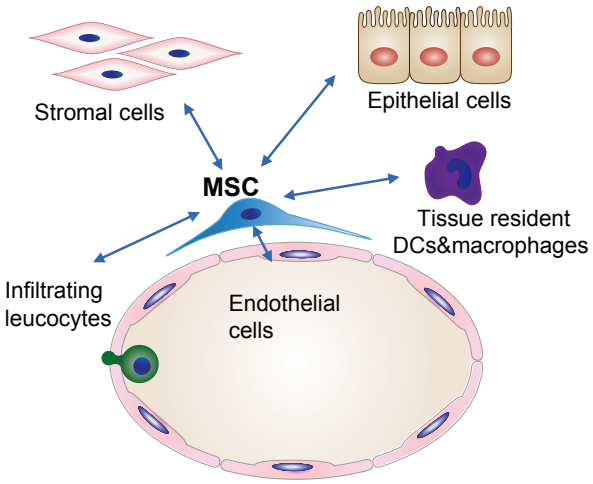


Figure 1. The central role of MSCs in tissue homeostasis. Due to the perivascular location of MSCs, MSCs are able to closely interact with several different cell types within an organ. (Adapted with permission from Leuning et al. Seminars in Nephrology 2014)

Little is still known about the exact mechanism of action of MSCs as cellular therapy. Upon activation of MSCs in an inflammatory milieu ('licensing'), MSCs express and excrete immunoregulatory molecules such as IDO and secrete several factors, including IL-6, TGF-β and prostaglandinE2 which are able to polarize monocytes towards immunosuppressive M2 macrophages and favour the induction of regulatory T cells. Moreover, macrophages that phagocytose MSCs are polarized towards the immunosuppressive M2 phenotype as summarized in figure 2 [2,5-10]. In several experimental models of kidney disease and transplantation, MSC treatment was able to enhanced tissue repair and reduce fibrosis as reviewed in a meta-analysis elsewhere[11].

In renal transplantation, the first clinical trials have demonstrated that MSC therapy is safe and feasible [12-15]. Currently several ongoing trials focus on the effects of MSCs on rejection, ischemia reperfusion injury, mimimization of immunosuppressive therapies and improvement of long-term graft survival[16].

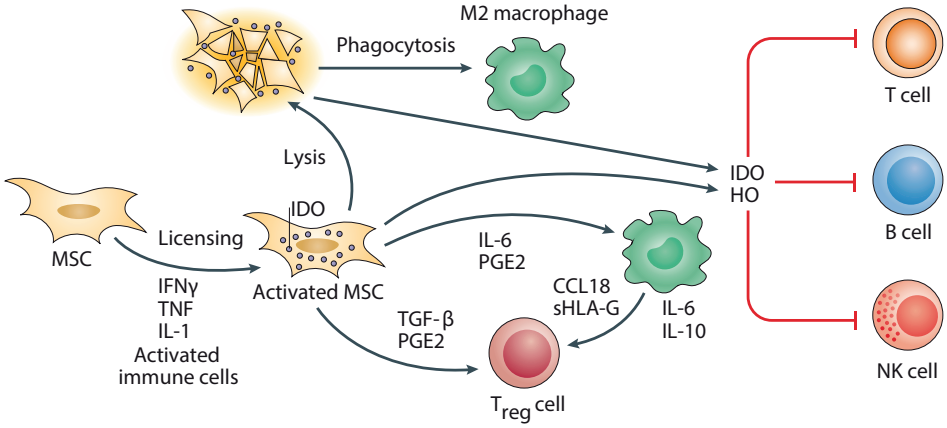


Figure 2. Putative immunomodulatory mechanism of action of mesenchymal stromal cells (MSCs). MSCs are short-lived and might be lysed soon after being injected into the circulation. Macrophages that phagocytose lysed MSCs are polarized to the immunosuppressive M2 phenotype. Activation or licensing by the proinflammatory microenvironment in the host (via licensing factors such as interferon γ (IFNγ), tumour necrosis factor (TNF) and IL1 or via direct interaction with activated immune cells), induces MSCs to express and secrete immunomodulatory molecules, such as indoleamine 2,3dioxygenase (IDO) and haemoxygenase (HO). These molecules have a role in suppressing the proliferation of target cells, including T cells, B cells and natural killer (NK) cells. Activated MSCs also induce polarization of monocytes towards immunosuppressive M2 macrophages via factors that include IL6 and prostaglandin E2 (PGE2). These macrophages secrete factors that contribute to the immunosuppressive state (including IL10 and IL6). They also produce CC chemokine ligand 18 (CCL18) and soluble HLAG (sHLAG), which in conjunction with MSC-derived factors, including transforming growth factor β (TGFβ) and PGE2, favour the induction of regulatory T (T_{reg}) cells. (Reprinted with permission from Fibbe et al. Nature Reviews Nephrology 2017)

For cellular therapy, in general 1-2x10^6 MSCs/kg bodyweight are given. Since the bone marrow only contains 0.001-0.01% primary MSCs, significant ex vivo amplification and culture is needed. The current standard clinical grade cell culture method of MSCs consists of culture on cell culture plastic in flasks or in cell factories. However, this method is time consuming and, due to the need of clean room facilities, costly. Therefore, there is a growing interest in closed-system bioreactor culture. In these systems, cells are usually grown on microcarriers. However, little is known about how these differences in microenvironment influence the functionality of the cells[17,18].

Previously it has been shown that MSC-like cells can be isolated from most organs. These MSC-like cells are mainly isolated from the perivascular compartment and exhibit tissue specific properties [19,20]. Perivascular stromal cells could also be isolated from the human kidney[21,22]. Due

to tissue specific imprinting, kidney derived perivascular stromal cells may be more potent in kidney regeneration compared to other sources of MSCs and are therefore an interesting new cell source for clinical therapy[22].

A bio-engineered kidney

Another novel strategy to diminish the shortage of donor organs and the need for immunosuppressive therapies in the future is the development of an autologous bioengineered kidney. For this purpose there is a need for both cells and an instructive matrix for cell adherence and organization. To obtain such a matrix human or human size kidney can be decellularized in order to obtain the extracellular matrix without the cells (scaffold). This scaffold can then be recellularized with induced pluripotent stem cells (hiPSCs) derived kidney- and endothelial cells derived from the patient.

First steps towards this bioengineered kidney have been made. Song et al. showed that when a rat kidney scaffold is recellularized with HUVEC and neonatal rat kidney cells, site specific recellularization was observed[23]. Moreover, others showed that rat kidney scaffolds can be recellularized with mouse embryonic stem cells which showed some differentiation into endothelial cells[24-28]. Although these studies are interesting first proof-of-concepts of a bioengineered kidney, all these studies were performed with either murine, porcine or kidney scaffolds and cells or with cell lines and are therefore not directly translatable to the human situation.

In order to develop a human bioengineered kidney, human or human size scaffolds should be recellularized with large quantities of human kidney- and endothelial cells preferably derived from the patient himself to avoid an immune reaction after transplantation. For these purposes human induced pluripotent stem cells (hiPSCs) would be the most attractive candidate as patient derived iPSCs can be expanded and differentiated into both kidney progenitor cells and endothelial cells[29,30]. Human or human size kidney scaffolds can then be recellularized with these iPSC-derived kidney and endothelial cells as schematically shown in figure 3.

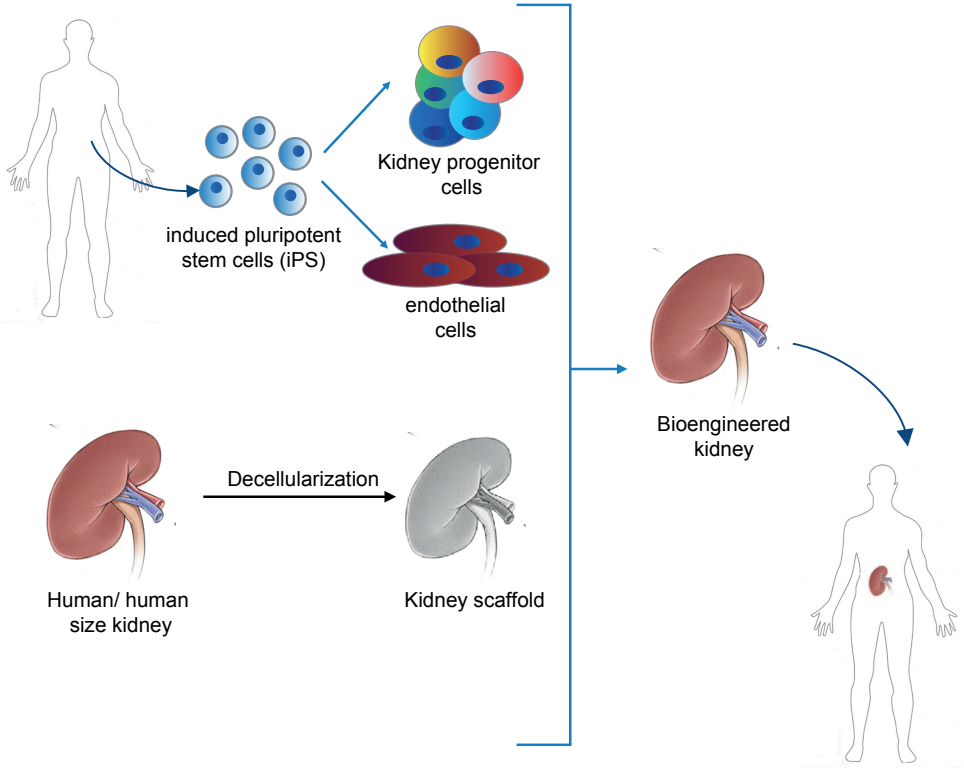


Figure 3. The concept of a bioengineered kidney by recellularizing a kidney scaffold with patient derived differentiated induced pluripotent stem cells.

Aims and outline of this thesis

The aim of this thesis was to explore novel therapeutic strategies for kidney transplantation in the field of regenerative medicine. In **chapter 2** latest insights, first clinical experiences and future perspectives and challenges of MSC therapy for kidney transplantation are discussed. At the moment, the focus of MSC therapy is mostly on bmMSCs. However, more and more evidence is arising that organ derived perivascular stromal cells exhibit MSC-like characteristics while at the same time they show tissue specific properties and reparative functions [19,20].

In **chapter 3** we show that kidney perivascular stromal cells (kPSCs) can be isolated from the human kidney and that these cells show, in contrast to bmMSCs, a organotypic expression signature and kidney specific regeneration properties. As we chose to focus on kPSCs as a novel candidate for cellular therapy in a clinical setting, we isolated these cells with a novel clinical grade isolation method of which the detailed protocol can be found in **chapter 4**.

As the culture of both kPSCs and bmMSCs in a clinical grade manner is currently time consuming and costly, culture methods are now shifting towards bioreactor-based systems where cells are cultured on microcarriers. However, little is known about how these changes in microenvironment influence the functionality of the cells. In **chapter 5** we investigated whether the microenvironment, specifically the topography of the culture surface, influence the secretome and thus functionality, of both kPSCs and bmMSCs.

Next, we show that within the human kidney, stromal cells can not only be found in the kidney cortex but also within the kidney capsule. These capsule derived MSCs show distinct gene expression profiles and functionality compared to cortex derived kPSCs. This underpins the large functional diversity of phenotypic similar stromal cells in relation to their anatomic site, even within one organ (**chapter 6**).

All MSC types studied above could potentially be able to prolong transplant survival, promote kidney regeneration and reduce the amounts of immunosuppressive therapies. However, in the most ideal situation immunosuppressive therapies would not be necessary at all. In this scenario, an ESRD patient would be transplanted with an autologous kidney made from differentiated patient-derived induced pluripotent stem cells. In **chapter 7** we show first critical steps towards this bioengineered kidney with the focus on the kidney vasculature.

Finally, **chapter 8** provides a general summary of the research presented in this thesis and further discusses the potentials of regenerative medicine for kidney diseases and transplantation.

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