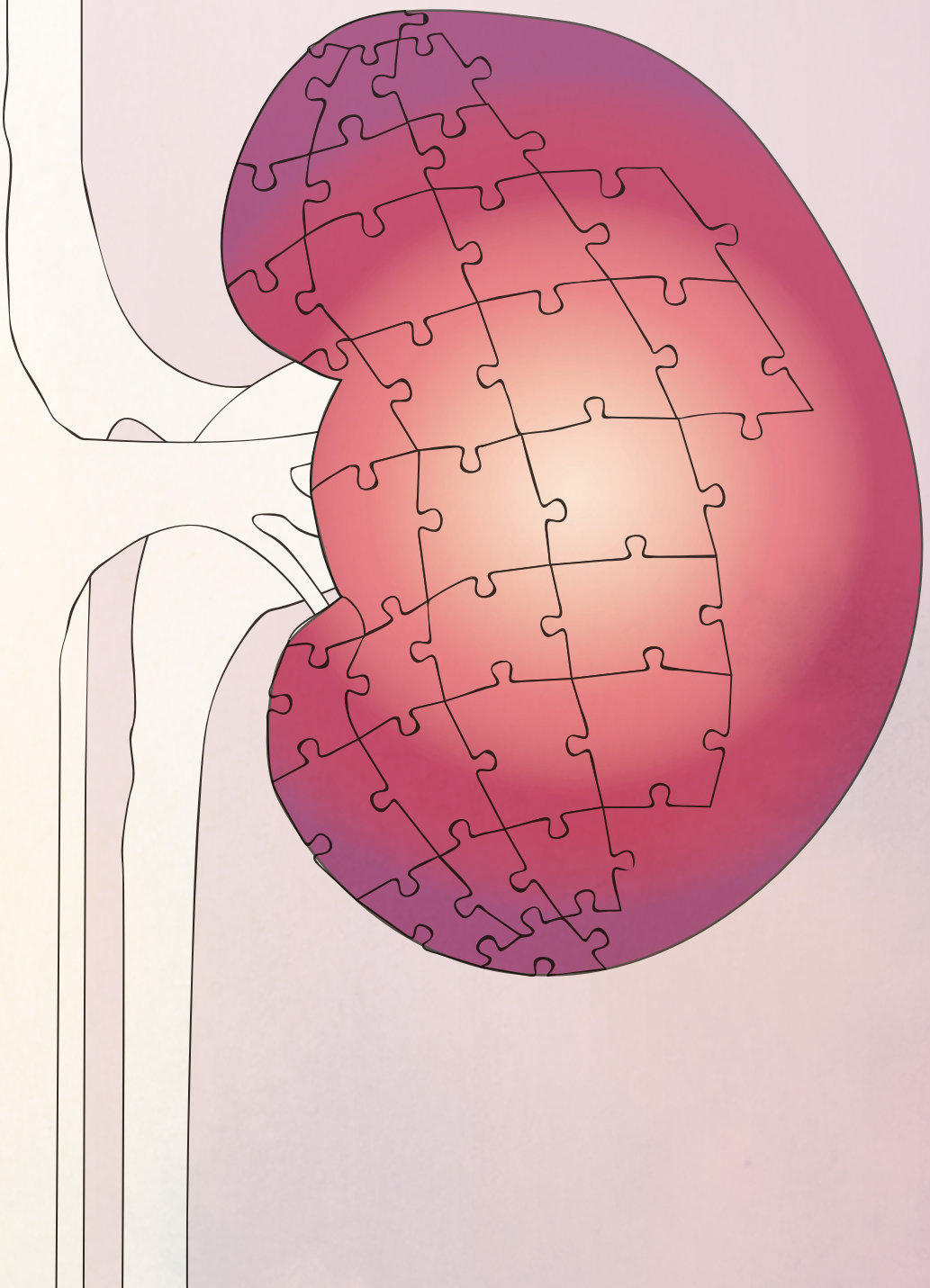
# Development of novel strategies to regenerate the human kidney

Leuning, D.G.

# Chapter 8
Summary and discussion



## Summary

The incidence of chronic kidney disease (CKD) and end stage renal disease (ESRD) is rising each year[1]. Kidney transplantation is currently the best therapy for patients with ESRD. However, there is a shortage of donor organs and the long term outcomes after kidney transplantation are compromised by the effects of rejection and nephrotoxicity of immunosuppressive therapies. There is therefore a need for new strategies to either prolong the survival of transplanted organs or to increase the numbers of (bio-engineered) kidneys suitable for transplantation.

Mesenchymal stromal cell (MSC) therapy is an interesting novel approach to increase transplant survival as MSCs exhibit antifibrotic and immunomodulatory properties. MSCs may therefore play roles in the treatment of allograft rejection and fibrosis and in minimization of immunosuppressive therapies, in particular calcineurin inhibitors. In several preclinical studies bone marrow MSCs (bmMSCs) showed beneficial effects on renal function and graft survival[2]. The first clinical studies have been performed with bmMSCs in kidney transplantation, mainly focussing on safety and feasibility[3-9]. Currently, several clinical trials are on-going focussing on improving long-term transplant survival with minimization of immunosuppression, prevention of transplant rejection or reducing ischemia reperfusion injury. While the first results are promising, there are, however, still a lot of questions which should be addressed regarding, amongst others, monitoring after MSC infusion, mechanism of action and optimal timing, dosage and frequency of infusions (**Chapter 2)**.

Mesenchymal stromal cells are a heterogeneous cell population and can be isolated from the perivascular fraction of most organs, including the human kidney (hkPSCs)[10-12]. In **chapter 3** we show an extensive characterization of kPSCs compared to bmMSCs and show that hkPSCs contain strong transcriptional similarities compared to bmMSCs, but also show organotypic expression signatures, including the HoxD10 and HoxD11 nephrogenic transcription factors. Comparable to bmMSCs, hkPSCs showed immunosuppressive potential and, when co-cultured with endothelial cells, vascular plexus formation was supported which was specifically in the hkPSCs accompanied by an increased NG2 expression. hkPSCs did not undergo myofibroblast transformation after exposure to TGFβ, further corroborating their potential regulatory role in tissue homeostasis. This was further supported by the observation that hkPSCs induced accelerated repair in a tubular epithelial wound scratch assay which was mediated through HGF release. *In vivo,* in a neonatal kidney injection model, hkPSCs re-integrated and survived in the interstitial compartment, while bmMSCs did not show this potential. Moreover, hkPSCs gave

protection against the development of acute kidney injury i*n vivo* in a model of rhabdomyolysis mediated nephrotoxicity. Overall, this suggests a superior therapeutic potential for the use of hkPSCs and/or their secretome in the treatment of kidney diseases.

For fluent clinical translation, we developed a clinical grade acceptable standard operation procedure (SOP) with the use of clinical grade materials and enzymes (**chapter 4)**.

As both the culture of 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[13-16]. 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 functionality of both kPSCs and bmMSCs. To this end, we cultured human bmMSCs and kPSCs in the TopoWell plate, a custom-fabricated multi well-plate containing 76 unique bioactive surface topographies. Using fluorescent imaging, we observed profound changes in cell shape, accompanied by major quantitative changes in the secretory capacity of the MSCs. The cytokine secretion profile was closely related to cell morphology which was influenced by the cell culture topography. Our data demonstrate that stromal cell function is determined by microenvironment structure and can be manipulated in an engineered setting. Our data also have implications for the clinical manufacturing of mesenchymal stromal cells, where surface topography during bioreactor expansion should be taken into account to preserve therapeutic properties.

Stromal cells from different organs show tissue-specific imprinting and properties[10,11,17-19]. However, the presence of functionally distinct stromal cell populations within one solid organ has not been described before. In **Chapter 6** we show that not only the kidney cortex but also the kidney capsule contains a stromal cell population. These capsule stromal cells are important for the three dimensional organisation of the kidney during nephrogenesis[20-25] and provide the barrier function of the capsule which is critical for homeostatic processes such as pressure natriuresis[26]. We postulated that stromal cells derived from the kidney capsule may therefore also have specific properties and functions. To this end, we isolated these capsule mesenchymal stromal cells (cMSC) from human cadaveric kidneys that were not suitable for transplantation. There were several similarities between cMSCs and kPSCs including support of vascular plexus formation, expression of phenotypic markers and resistance against myofibroblast transformation. However, compared to kPSCs, cMSCs showed distinct mRNA and miRNA expression profiles, showed increased immunosuppressive capacity, and displayed strongly reduced HGF production, contributing to the inability to enhance kidney epithelial repair.

Therefore cMSCs are a distinct, novel human kidney-derived MSC-population and these data underpin the large functional diversity of phenotypically similar stromal cells in relation to their anatomic site, even within one organ.

Although the in-organ differential functionality of MSCs is of interest to our understanding of the structural biology of the kidney, cMSCs are less likely to be used as candidate for clinical therapies. In clinical trials usually 2 cell infusions of 1-2 million cells per kilogram body weight are given[8]. To obtain such cells numbers, cMSCs should be isolated from several different donors for infusion into one patient, which makes the use of cMSCs less feasible compared to other MSC sources.

Next to MSC therapy to increase transplant survival, another future strategy to increase the numbers of available organs for transplantation may be kidney bio-engineering. For this purpose a human or human size kidney can be decellularized in order to obtain the kidney matrix without the cells (scaffold). This scaffold can then be recellularized with induced pluripotent stem cells (hiPSCs) derived kidney[27]- and endothelial cells[28] derived from the patient. In **chapter 7** we report the regeneration of kidney vasculature by repopulating the glomerular and peritubular vascular compartment of human and rat kidney matrices with human endothelial cells derived from human glomeruli and human induced pluripotent stem cells. In order to optimize re-endothelialization, we provide novel strategies such as growth factor loading and a new controlled arterio-venous delivery system. Using this novel approach we were able to scale up re-endothelialization using iPS-derived endothelial cells as an expandable source to a full human kidney matrix, and were able to achieve efficient cell delivery, adherence and survival of these endothelial cells as a first, but critical, step towards a human bioengineered kidney.

## Discussion and future perspectives



Already in Greek mythology there was a fascination for regeneration of organs and body parts. For example, the liver of Prometheus, destroyed every night by an eagle, regenerated every morning. Not only in mythology but also in early science there was an interest in regeneration as Aristotle (384-322 BC) already observed that a lizard is able to completely regenerate its lost tail[29-31].

Whereas the human body is not able to completely regenerate lost body parts, it does have a regenerative capacity. While some cell types, including kidney tubular epithelial cells, are able to self-renew[32,33], other nephron segments such as the glomerulus have a limited regenerative

capacity. Stromal cells are of particular importance for tissue regeneration as stromal cells can, due to their perivascular location and spindle shaped morphology, communicate with several cell types in the organ including endothelial cells, tissue resident macrophages and dendritic cells and infiltrating leucocytes[8,34]. Stromal cells are therefore an interesting cell source for cellular therapy for kidney regeneration and are currently extensively studied for kidney transplantation, both preclinically and in clinical studies. For clinical application, many questions regarding MSC therapy still remain, including the best MSC source, the most effective delivery strategy and long term effects and safety (**chapter 2).**

Little is known about the best MSC source for kidney transplantation patients. Most studies performed in the transplantation field have been performed with bmMSCs. However, as we show that bmMSCs do not produce substantial levels of HGF important for kidney regeneration (**chapter 3)**, bmMSCs may not be the best option in kidney transplantation. kPSCs do show this potential, but are not as readily available and need longer culture times and thus have a higher risk of infection and the acquiring of genetic anomalies during culture. Another option would be umbilical cord derived MSCs (ucMSCs). Within the STELLAR consortium we and others showed that ucMSCs have comparable kidney regeneration capacity *in vitro* and are able to ameliorate kidney damage *in vivo*[35]. As umbilical cords are easily obtained and ucMSCs can be isolated at low passage in high quantities, ucMSCs are an interesting cell source for further exploration for kidney transplantation.

Another issue to be resolved is the administration method of MSCs. Currently, MSCs are given intravenously to the patient[8]. However, it may be more potent to deliver the MSCs, or the MSC conditioned medium containing the factors important for kidney regeneration, to the kidney *ex vivo* before transplantation. This would decrease the potential risk of immunity, and potential long term negative effects of MSCs in the patient. Moreover, the MSC conditioned medium could be combined with *ex situ* machine perfusion or *in situ* regional perfusion resuscitation strategies[36] and may in such a way be able to enhance transplant regeneration.

Perivascular stromal cells are only a small fraction of the cell population in the bone marrow and kidney. Therefore *in vitro* expansion is necessary to obtain sufficient cell numbers. This is a time consuming and, particular in a clinical setting where clean room facilities are necessary, costly process. Moreover, open procedures increase the risk of infections during culture. Therefore there is a growing interest in closed-system bioreactors[13-16]. We and others showed that both bmMSCs and kPSCs can be cultured on biodegradable microcarriers in a xeno-free and serum-free spinnerflask culture[35]. Moreover, kPSCs could be isolated from the total kidney cell suspension via magnetic NG2 separation using fully automated cell processing (CliniMACS

Prodigy ®)[35]. Combining these two techniques would result in a closed system isolation and culture method, diminishing the hands-on time and infection risks. However, stromal cells cultured with these novel techniques should be extensively characterized as we and others showed that the functionality of stromal cells can be different when cells are cultured on different materials, surface shape and surface stiffness (**chapter 5**, [37-41]).

The field of regenerative medicine made enormous progress since the discovery in 2006 that differentiated adult cells can be reprogrammed into induced pluripotent stem cells (iPSC)[42]. In particular the combination of iPSC-technology with more recent differentiation strategies of iPSC towards several different cell types in the body, including neural[43], retinal pigment epithelial (RPE) cells[44], β-cells[45], kidney[27] and endothelial cells[28] make iPS-derived cells and tissues interesting new therapeutic strategies for regeneration. In fact, the first clinical study with autologous iPS-derived RPE cells for macular degeneration was initiated in 2014. Although currently on hold due to mutations observed in the second patient's iPSCs, it is anticipated to resume[46]. More trials are to be expected with iPS-derived cells.

Although the kidney is a complex organ, consisting of around 2 million nephrons containing more than 20 distinct cell types, it turned out to be possible to create iPS-derived nephrons in 3D structures (organoids) by mimicking embryonic development. These organoids show self-organization into nephrons containing glomeruli, proximal tubules, early loop of Henles, distal tubules and collecting ducts[27].

While these findings hold great potential, for example for drug screening purposes, the size of the kidney organoids at present is too small to use directly for renal replacement therapy. Moreover, nephron structures within the organoids lack a collecting system and a connected vasculature. Using the kidney extracellular matrix as an instructive guidance for iPS-derived kidney- and endothelial cells may be a method to overcome these hurdles towards a bio-engineered kidney (**chapter 7**), [47,48].

The concept of a bio-engineered kidney using the decellularized extracellular matrix is based on the theory that the instructive capacity of the matrix is site specific and results in site selective cell engraftment. We show that the instructive capacity can be used for enhanced human endothelial cell adherence and survival in whole organ kidney matrices based on the preservation of the glycosaminoglycan (GAG) landscape (**chapter 7**). To gain a functional bio-engineered organ, however, also the epithelial side and stromal compartment of the kidney should be re-cellularized. Song et al. showed that by recellularizing rat kidney matrices with rat neonatal kidney cells, indeed side selective engraftment was observed with podocytes in the glomerular structures and

tubular cells in the tubular compartment of the nephron[47]. However, whether this is also possible with iPS-derived kidney progenitor cells still needs to be further elucidated. Moreover, culture methods of iPSC-derived kidney progenitor cells should be optimized to enable the production of large cell quantities necessary to recellularized a human or human size kidney matrix.

Besides the hurdles mentioned above, there are still several other obstacles such as sterility, thrombogenicity and immunogenicity, which need to be resolved. The vascular compartment, for example, needs to be completely re-endothelialized to prevent thrombosis. Moreover, little is known about the immunogenicity of the scaffold after recellularization. A massive influx of immune cells is observed in a transplanted empty scaffold[48]. Whether this is also the case for transplanted re-cellularized scaffolds still needs to be further investigated.

Moreover, there are also still safety issues regarding iPSC for clinical application, such as genetically stability and risks of tumorigenicity[46]. In the end, direct reprogramming of adult fibroblasts into the desired cell type without a pluripotent state in between may be a safer option [49,50]. However, whether with this methods sufficient cell numbers of all cell types can be acquired is currently not known.

With all these hurdles in mind, it has to be seen whether a transplantable bio-engineered kidney will be possible in the future. But even when this will turn out to be unfeasible, a lot of novel knowledge about the instructiveness of the extracellular matrix is obtained along the way. This knowledge itself may provide important clues for organ regeneration *in situ.* In fact, recently it has been described that the method to obtain the decellularized matrix is feasible *in situ*[51]. One could think of a future were non-functional parts of an organ are decellularized *in* vivo and, combined with strategies to enhance cell adherence, proliferation and functionality, such as growth factor loading, can instruct the body itself to regenerate this matrix.

Not only in the scientific world, but also from a commercial perspective, the field of regenerative medicine is increasing. According to the World Regenerative Medicines Market forecast 2015-2022 the field of regenerative medicine is projected to reach $30,237 million by 2022, mainly in the field of cellular therapies[52] . Unfortunately, the increasing interest in the field and resulting potential gains also has its drawbacks. There is a growing industry in stem cell tourism based on unproven treatments and several allegations of research misconduct have been reported[31,53-55]. As there are currently still major issues to be resolved regarding iPS- derived product safety, such as genetically stability and risks of tumorigenicity[46], it is of importance that the general public, in particular the patients, get a realistic view about the possibilities of stem cell therapies. The research community has a major responsibility in this in their communication with the media.

To conclude, the field of regenerative medicine holds, despite the mentioned hurdles, a lot of promises for kidney transplantation on both the short and long term. However, time will tell whether all aspects, including iPS-based therapies and bio-engineered organs, will be clinically available in the future or will be a modern myth.

# References


1 Levey, A. S. & Coresh, J. Chronic kidney disease. *Lancet.* **379** (9811), 165-180.

2 Wang, Y., He, J., Pei, X. & Zhao, W. Systematic review and meta-analysis of mesenchymal stem/stromal cells therapy for impaired renal function in small animal models. *Nephrology (Carlton).* **18** (3), 201-208, doi:10.1111/nep.12018, (2013).

3 Reinders, M. E. *et al.* Autologous bone marrow-derived mesenchymal stromal cells for the treatment of allograft rejection after renal transplantation: results of a phase I study. *Stem Cells Transl Med.* **2** (2), 107-111, doi:10.5966/sctm.2012-0114sctm.2012-0114 [pii], (2013).

4 Perico, N. *et al.* Autologous mesenchymal stromal cells and kidney transplantation: a pilot study of safety and clinical feasibility. *Clin J Am Soc Nephrol.* **6** (2), 412-422 (2011).

5 Perico, N. *et al.* Mesenchymal stromal cells and kidney transplantation: pretransplant infusion protects from graft dysfunction while fostering immunoregulation. *Transpl Int.* **26** (9), 867-878, doi:10.1111/tri.12132, (2013).

6 Tan, J. *et al.* Induction therapy with autologous mesenchymal stem cells in living-related kidney transplants: a randomized controlled trial. *JAMA.* **307** (11), 1169-1177, doi:10.1001/jama.2012.316307/11/1169 [pii], (2012).

7 Peng, Y. *et al.* Donor-derived mesenchymal stem cells combined with low-dose tacrolimus prevent acute rejection after renal transplantation: a clinical pilot study. *Transplantation.* **95** (1), 161-168, doi:10.1097/TP.0b013e318275 4c5300007890-201301150-00024 [pii], (2013).

8 Leuning, D. G., Reinders, M. E., de Fijter, J. W. & Rabelink, T. J. Clinical translation of multipotent mesenchymal stromal cells in transplantation. *Semin Nephrol.* **34** (4), 351-364, doi:10.1016/j.semnephrol.2014.06.002, (2014).

9 Reinders, M. E. J., van Kooten, C., Rabelink, T. J. & de Fijter, J. W. Mesenchymal Stromal Cell Therapy for Solid Organ Transplantation. *Transplantation.* doi:10.1097/TP.0000000000001879, (2017).

10 Crisan, M. *et al.* A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell.* **3** (3), 301-313 (2008).

11 Leuning, D. G. *et al.* Clinical-Grade Isolated Human Kidney Perivascular Stromal Cells as an Organotypic Cell Source for Kidney Regenerative Medicine. *Stem Cells Transl Med.* doi:10.5966/sctm.2016-0053, (2016).

12 Bruno, S. *et al.* Isolation and characterization of resident mesenchymal stem cells in human glomeruli. *Stem Cells Dev.* **18** (6), 867-880, doi:10.1089/scd.2008.0320, (2009).

13 Carmelo, J. G., Fernandes-Platzgummer, A., Diogo, M. M., da Silva, C. L. & Cabral, J. M. A xeno-free microcarrier-based stirred culture system for the scalable expansion of human mesenchymal stem/stromal cells isolated from bone marrow and adipose tissue. *Biotechnol J.* **10** (8), 1235-1247, doi:10.1002/biot.201400586, (2015).

14 Fernandes-Platzgummer, A., Carmelo, J. G., da Silva, C. L. & Cabral, J. M. Clinical-Grade Manufacturing of Therapeutic Human Mesenchymal Stem/Stromal Cells in Microcarrier-Based Culture Systems. *Methods Mol Biol.* **1416** 375-388, doi:10.1007/978-1-4939-3584-0_22, (2016).

15 Lam, A. T. *et al.* Biodegradable poly-epsilon-caprolactone microcarriers for efficient production of human mesenchymal stromal cells and secreted cytokines in batch and fed-batch bioreactors. *Cytotherapy.* **19** (3), 419-432, doi:10.1016/j.jcyt.2016.11.009, (2017).

16 Mizukami, A. *et al.* Stirred tank bioreactor culture combined with serum-/xenogeneic-free culture medium enables an efficient expansion

of umbilical cord-derived mesenchymal stem/stromal cells. *Biotechnol J.* **11** (8), 1048-1059, doi:10.1002/biot.201500532, (2016).

17 Chen, W. C. *et al.* Human myocardial pericytes: multipotent mesodermal precursors exhibiting cardiac specificity. *Stem Cells.* **33** (2), 557-573, doi:10.1002/stem.1868, (2015).

18 Naftali-Shani, N. *et al.* The origin of human mesenchymal stromal cells dictates their reparative properties. *J Am Heart Assoc.* **2** (5), e000253, doi:10.1161/JAHA.113.000253, (2013).

19 Li, J. *et al.* Collecting duct-derived cells display mesenchymal stem cell properties and retain selective in vitro and in vivo epithelial capacity. *J Am Soc Nephrol.* **26** (1), 81-94, doi:10.1681/asn.2013050517, (2015).

20 Boivin, F. J., Sarin, S., Evans, J. C. & Bridgewater, D. The Good and Bad of beta-Catenin in Kidney Development and Renal Dysplasia. *Front Cell Dev Biol.* **3** 81, doi:10.3389/fcell.2015.00081, (2015).

21 Boivin, F. J. *et al.* Stromally expressed beta-catenin modulates Wnt9b signaling in the ureteric epithelium. *PLoS One.* **10** (3), e0120347, doi:10.1371/journal.pone.0120347, (2015).

22 Li, W., Hartwig, S. & Rosenblum, N. D. Developmental origins and functions of stromal cells in the normal and diseased mammalian kidney. *Dev Dyn.* **243** (7), 853-863, doi:10.1002/dvdy.24134, (2014).

23 Little, M. H., Combes, A. N. & Takasato, M. Understanding kidney morphogenesis to guide renal tissue regeneration. *Nat Rev Nephrol.* **12** (10), 624-635, doi:10.1038/nrneph.2016.126, (2016).

24 Levinson, R. S. *et al.* Foxd1-dependent signals control cellularity in the renal capsule, a structure required for normal renal development. *Development.* **132** (3), 529-539, doi:10.1242/dev.01604, (2005).

25 Yallowitz, A. R., Hrycaj, S. M., Short, K. M., Smyth, I. M. & Wellik, D. M. Hox10 genes function in kidney development in the differentiation and integration of the cortical stroma. *PLoS One.* **6** (8), e23410, doi:10.1371/journal.pone.0023410, (2011).

26 Garcia-Estan, J. & Roman, R. J. Role of renal interstitial hydrostatic pressure in the pressure diuresis response. *Am J Physiol.* **256** (1 Pt 2), F63-70 (1989).

27 Takasato, M. *et al.* Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. *Nature.* **526** (7574), 564-568, doi:10.1038/nature15695, (2015).

28 Orlova, V. V. *et al.* Generation, expansion and functional analysis of endothelial cells and pericytes derived from human pluripotent stem cells. *Nat Protoc.* **9** (6), 1514-1531, doi:10.1038/nprot.2014.102, (2014).

29 Aristotle. Historia Animalium. *In: Peck AL, translator. Vol 1. Cambridge: Harvard University Press; 1965. 239 pp.*

30 Odelberg, S. J. Unraveling the molecular basis for regenerative cellular plasticity. *PLoS Biol.* **2** (8), E232, doi:10.1371/journal.pbio.0020232, (2004).

31 Jessop, Z. M., Al-Sabah, A., Francis, W. R. & Whitaker, I. S. Transforming healthcare through regenerative medicine. *BMC Med.* **14** (1), 115, doi:10.1186/s12916-016-0669-4, (2016).

32 Kusaba, T., Lalli, M., Kramann, R., Kobayashi, A. & Humphreys, B. D. Differentiated kidney epithelial cells repair injured proximal tubule. *Proc Natl Acad Sci U S A.* **111** (4), 1527-1532, doi:10.1073/pnas.1310653110, (2014).

33 Chang-Panesso, M. & Humphreys, B. D. Cellular plasticity in kidney injury and repair. *Nat Rev Nephrol.* **13** (1), 39-46, doi:10.1038/nrneph.2016.169, (2017).

34 Schepers, K. & Fibbe, W. E. Unraveling mechanisms of mesenchymal stromal cell-mediated immunomodulation through patient monitoring and product characterization. *Ann N Y Acad Sci.* **1370** (1), 15-23, doi:10.1111/nyas.12984, (2016).

35 FP-7-Health-2012 STELLAR.

36 Jochmans, I. *et al.* Past, Present, and Future of Dynamic Kidney and Liver Preservation and Resuscitation. *Am J Transplant.* **16** (9), 2545-2555, doi:10.1111/ajt.13778, (2016).

37 Seib, F. P., Prewitz, M., Werner, C. & Bornhauser, M. Matrix elasticity regulates the secretory profile of human bone marrow-derived multipotent mesenchymal stromal cells (MSCs). *Biochem Biophys Res Commun.* **389** (4), 663-667, doi:10.1016/j.bbrc.2009.09.051, (2009).

38 Zhou, S. B., Wang, J., Chiang, C. A., Sheng, L. L. & Li, Q. F. Mechanical stretch upregulates SDF-1alpha in skin tissue and induces migration of circulating bone marrow-derived stem cells into the expanded skin. *Stem Cells.* **31** (12), 2703-2713, doi:10.1002/stem.1479, (2013).

39 Wang, D. L. *et al.* Mechanical strain induces monocyte chemotactic protein-1 gene expression in endothelial cells. Effects of mechanical strain on monocyte adhesion to endothelial cells. *Circ Res.* **77** (2), 294-302 (1995).

40 Quinn, T. P., Schlueter, M., Soifer, S. J. & Gutierrez, J. A. Cyclic mechanical stretch induces VEGF and FGF-2 expression in pulmonary vascular smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol.* **282** (5), L897-903, doi:10.1152/ajplung.00044.2001, (2002).

41 Mammoto, A. *et al.* A mechanosensitive transcriptional mechanism that controls angiogenesis. *Nature.* **457** (7233), 1103-1108, doi:10.1038/nature07765, (2009).

42 Takahashi, K. & Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell.* **126** (4), 663-676, doi:10.1016/j.cell.2006.07.024, (2006).

43 Pasca, A. M. *et al.* Functional cortical neurons and astrocytes from human pluripotent stem cells in 3D culture. *Nat Methods.* **12** (7), 671-678, doi:10.1038/nmeth.3415, (2015).

44 Croze, R. H. & Clegg, D. O. Differentiation of pluripotent stem cells into retinal pigmented epithelium. *Dev Ophthalmol.* **53** 81-96, doi:10.1159/000357361, (2014).

45 Abdelalim, E. M. & Emara, M. M. Advances and challenges in the differentiation of pluripotent stem cells into pancreatic beta cells. *World J Stem Cells.* **7** (1), 174-181, doi:10.4252/wjsc.v7.i1.174, (2015).

46 Shi, Y., Inoue, H., Wu, J. C. & Yamanaka, S. Induced pluripotent stem cell technology: a decade of progress. *Nat Rev Drug Discov.* **16** (2), 115-130, doi:10.1038/nrd.2016.245, (2017).

47 Song, J. J. *et al.* Regeneration and experimental orthotopic transplantation of a bioengineered kidney. *Nat Med.* **19** (5), 646-651, doi:10.1038/nm.3154, (2013).

48 Remuzzi, A. *et al.* Experimental Evaluation of Kidney Regeneration by Organ Scaffold Recellularization. *Sci Rep.* **7** 43502, doi:10.1038/srep43502, (2017).

49 Fu, J. D. *et al.* Direct reprogramming of human fibroblasts toward a cardiomyocyte-like state. *Stem Cell Reports.* **1** (3), 235-247, doi:10.1016/j.stemcr.2013.07.005, (2013).

50 Hendry, C. E. *et al.* Direct transcriptional reprogramming of adult cells to embryonic nephron progenitors. *J Am Soc Nephrol.* **24** (9), 1424-1434, doi:10.1681/ASN.2012121143, (2013).

51 Mayorca-Guiliani, A. E. *et al.* ISDoT: in situ decellularization of tissues for high-resolution imaging and proteomic analysis of native extracellular matrix. *Nat Med.* **23** (7), 890-898, doi:10.1038/nm.4352, (2017).

52 Regenerative Medicines Market by technology (Cell Therapy, Gene Therapy, Tissue Engineering, Small Molecules & Biologics), Material (Biodegradable Synthetic Polymers, Scaffold, Hydrogel & Collagen, Transgenic, Fibroblasts, Small Molecules and Biologics) - Global Opportunity Analysis and Industry Forecast, 2015 - 2022. (2017).

53 Cyranoski, D. Acid bath offers easy path to stem cells. *Nature.* **505** (7485), 596, doi:10.1038/505596a, (2014).

54 Charo, R. A. On the Road (to a Cure?)--Stem-Cell Tourism and Lessons for Gene Editing. *N Engl J Med.* **374** (10), 901-903, doi:10.1056/NEJMp1600891, (2016).

55 Matthews, K. R. & Iltis, A. S. Unproven stem cell-based interventions and achieving a compromise policy among the multiple stakeholders. *BMC Med Ethics.* **16** (1), 75, doi:10.1186/s12910-015-0069-x, (2015).