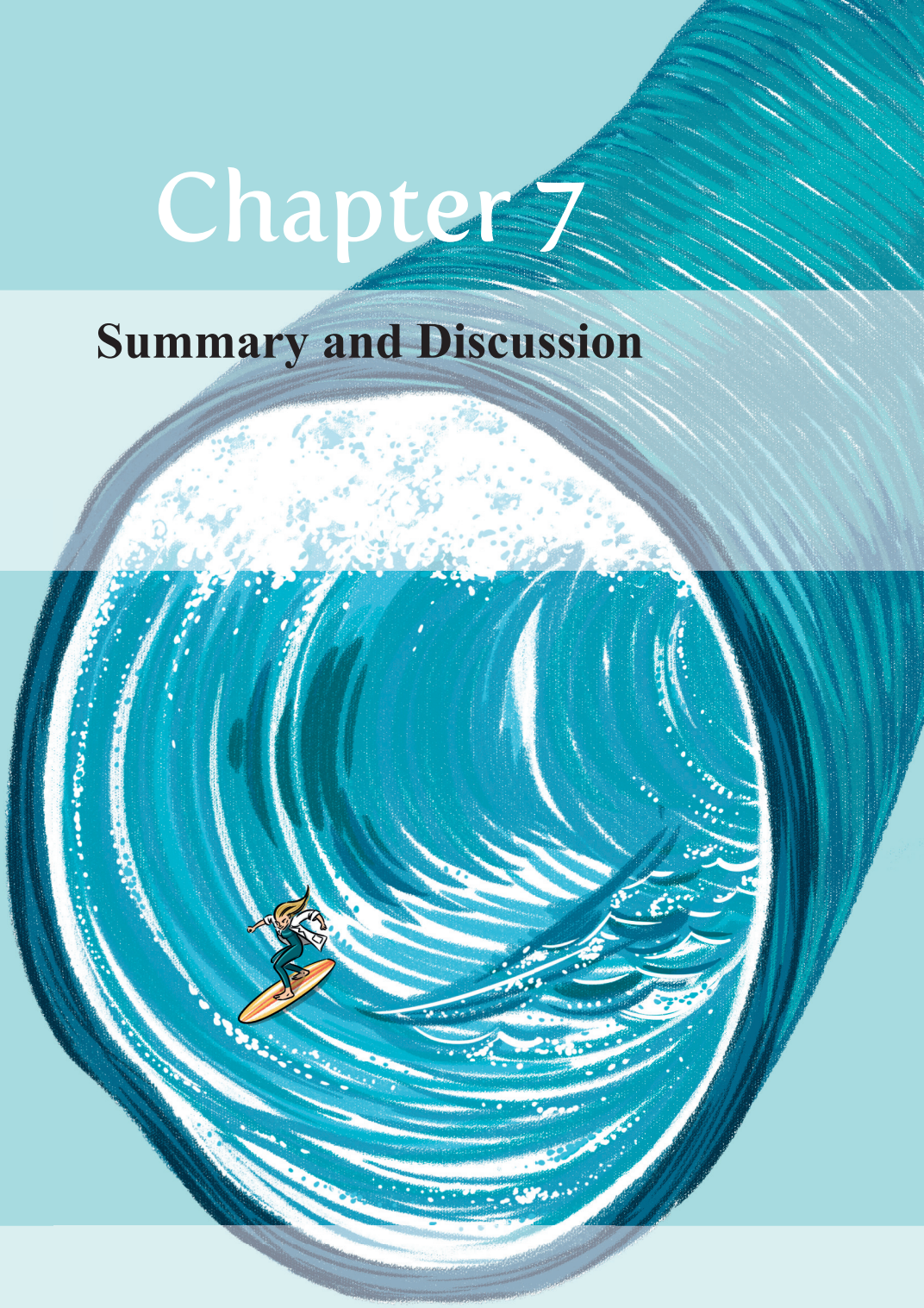
Hemodialysis vascular access failure: novel pathophysiological mechanisms and therapeutic strategies

Bezhaeva, T.

Chapter 7

Summary and Discussion



How it all started?

The view on renal replacement therapy was revolutionized in 1943 when the Dutch physician Dr. Willem Kollf built a first prototype of a dialyzer[1,2]. The first dialysis machines could get acute renal failure patients through a crisis until renal recovery, but the Achilles heel was a reliable access to the circulation for multiple dialysis sessions, which did not yet exist. Dialysis therapy for ESRD was first successfully attempted by Scribner in 1960 (Seatle, USA) after Quinton built the first shunt, based on a teflon-siliac loop externally placed between artery and vein of a patient[3]. Clyde Shields, a Boeing machinist, survived for 11 years after the insertion of Scribner's first AV shunt on 9 March 1960. Yet, Scribner-Quinton shunts usually lasted a few months or less, and were prone to clotting, skin necrosis, bleeding, and infection.

James E. Cimino and Michael J. Brescia (New York, USA) first described a 'simple venipuncture for hemodialysis'[4] followed by the historical paper 'Chronic hemodialysis using venipuncture and a surgically created arteriovenous fistula'[5] published in 1966 in NEJM. Dr. Cimino says. "We were bold in using a procedure that had always been considered physiologically abnormal, but without adequate vascular access our patients were doomed." Later, recalling the development of the AV fistula, Dr. Cimino adds "I had no idea our technique would continue to be popular for so many years later. I thought the real advances were going to be in chemistry, and that scientists would develop a pill to help patients with end-stage renal disease".

Where do we stand right now?

Even though at present kidney transplantation has become the preferred modality in kidney replacement therapy, the shortage of suitable donors is still persist. A considerable 40% of ESRD patients still depend on dialysis[6]. An adequately functioning AVF, described by Brescia and Cimino, remains the first choice for chronic HD serving as a lifeline for a lifetime in hemodialysis patients. Up to now, international guidelines strongly encourage the creation of AVFs for HD vascular access over prosthetic AV grafts and CVCs[7]. Ironically, being preferred and most commonly used vascular access point, AVFs dysfunction remains a major source of morbidity for patients with ESRD. At current, only few effective therapies for this clinical problem are available. One of the reasons is limited understanding of the underlying mechanisms that lead to AVF failure. The availability of animal models made it possible to study the pathogenesis of vascular access failure and to evaluate new treatment candidates.

Although the exact pathophysiology of AVF failure is not completely understood, there is a general consensus between researchers that IH and inadequate OR are two main processes that contribute to AVF failure[8,9]. Recent studies have shown that the process of vascular adaptation is mainly driven by altered hemodynamics after AVF creation and is associated with an excessive inflammatory response and proliferation and migration of arterial and venous VSMCs towards the intima at the site of anastomosis[10-14].

The work described in this thesis aimed to further understand the pathobiology of

vascular access dysfunction and identify new therapeutic candidates to improve the current status of hemodialysis vascular access.

New insights into the pathobiology of AVF

Targeting inflammation in AVF maturation

Although several studies have shown that the adaptive response upon AVF creation triggers infiltration of macrophages and lymphocytes[11] as well as upregulation of pro-inflammatory cytokine production[14], the role of inflammation in vascular remodeling upon AVF surgery has not been unraveled. Based on the previous knowledge that the TLR4 mediated inflammatory response contributes to several vascular pathologies[15-17], in **chapter 2** of this thesis we addressed the specific role of TLR4 homologue RP105 in vascular remodeling, inflammation and VSMCs function in a murine model of AVF failure. We clearly show that RP105 deficiency affects the inflammatory and VSMC-mediated response to injury during the course of AVF maturation, accumulating in an impaired outward remodeling of the venous outflow track of the AVF.

More detailed analysis of the effects of RP105 deficiency on the inflammatory response revealed strong accumulation of anti-inflammatory macrophages and an unexpected decrease in pro-inflammatory macrophages. The dominance of anti-inflammatory macrophages (> 90% of total macrophages) in the lesions at 2 weeks after AVF creation suggest either that in the current model pro-inflammatory response is completed at earlier time points, or that anti-inflammatory macrophages play a dominant role in the tissue response in murine AVF. Unfortunately, we did not study macrophage subpopulations at earlier time points. Concurrently, we observed a 50% decrease in MMP-activity *in vivo*, suggesting that the attenuation of vessel wall MMP activity limits venous OR in maturating AVFs.

VSMC content in AVFs from RP105 deficient mice was markedly decreased. *In vitro*, proliferation of venous VSMCs from RP105 deficient mice was reduced by 50%, whereas arterial VSMCs displayed a 50% decrease in migration. While VSMC proliferation in IH is generally considered to be detrimental, the process might be beneficial for OR, especially in the early phase of AVF maturation. In this respect, the observed reduction in venous outward remodeling, coupled with a reduction in proliferating venous VSMCs within AVFs, suggests that new drug targets designed to inhibit VSMCs proliferation could be detrimental for AVF maturation.

A striking observation in our studies was that RP105 diminution differentially affected arterial and venous VSMCs, as evidenced by RP105-specific effects on migration, proliferation and inflammatory cytokine production that differed substantially between these two cell sources. The endogenous expression levels of RP105 in arterial and venous VSMCs support this finding, along with differential expression profiles of associating TLR4-family members (including TLR4 and MD1). These findings illustrate the need for continued investigation of the phenotypic properties and functional characteristics of VSMCs in AVFs, in particular due to the contrasting lineage tracing studies detailing a predominance of arterial VSMCs[18] versus venous VSMCs[19] in venous IH following

AVF placement. This issue is further discussed in **chapter 5** of this thesis.

Overall, results of this study demonstrate that deletion of TLR4 homologue RP105 trigger alterations on various cell types involved in AVF maturation. In order to improve AVF outcomes, future therapeutic interventions targeting the TLR4/RP105 axis must include time-dependent and cell specific targeting approaches to steer the vascular remodeling response in the proper direction.

Our next step in understanding the role of inflammation in AVF maturation was aimed to understand whether inhibition of early inflammatory response triggered by AVF surgery directly contributes to maturation failure. Glucocorticoids (GCs) are well known anti-inflammatory drugs that bind to cytosolic glucocorticoid receptors in target cells, leading to the down-regulation in inflammatory cytokine production and reduction in inflammatory cell recruitment[20,21]. Despite being potent anti-inflammatory drugs chronic and systemic use of GCs is limited due to high incidents of severe side-effects[22]. In **chapter 3** of this thesis we aimed to test whether administration of liposomal prednisolone (L-Pred) will result in local inhibition of inflammatory response and, consequently, better AVF maturation. Targeted drug delivery achieved by incorporation of prednisolone into the liposomes hold great potential. As a result of enhanced vascular permeability caused by AVF surgery, circulating liposomal nanoparticles will extravasate and accumulate in the area around AV-anastomosis. To maximize efficacy and minimize toxicity of prednisolone is extremely important in such vulnerable patients as with ESRD.

First, using near infrared microscopy we demonstrated that liposomes extravasate in macrophages in the post-anastomotic area of the venous outflow tract—the most prone area of IH formation. As expected, we observed an 83% reduction in infiltrating leukocytes from which subpopulation of T-lymphocytes and granulocytes were reduced by 86% and 51% respectively. Treatment of macrophages with L-Pred *in vitro* induced transition towards an anti-inflammatory phenotype. Overall shift towards anti-inflammatory state might have inhibited the recruitment of lymphocytes and granulocytes to the AV-anastomosis. Alternatively, release of prednisolone from macrophages might have had a direct effect on lymphocytes and granulocytes.

At a morphometrical level, treatment with L-Pred resulted in a 27% increase in outward remodeling and 47% increase in luminal area. Control mice treated with unencapsulated prednisolone or liposomes loaded with PBS did not show any effect on morphometry. The exact mechanism by which liposomal prednisolone resulted in increased OR in murine AVF is not clear. We speculate that matrix metalloproteinases might contribute to this vascular response. The effect of MMPs on the vascular remodeling depend on the specific MMPs that are activated and the type of vascular injury[23]. Elegant study by Nieves Torres and coworkers[24] suggests effect of MMP inhibition on venous outward remodeling in AVF. Indeed, adventitial delivery of a small hairpin RNA against the MMP ADAMTS-1, resulted in reduced macrophage infiltration, decreased MMP9 activity and enhanced outward remodeling in murine AVF.

In our study, *in vitro* treatment of macrophages with L-Pred resulted in decreased MMP2 and MMP9 gene expression. Although we were not able to quantify MMP activity *in vivo*, we speculate that enhanced outward remodeling upon L-Pred treatment is mediated by the inhibition of inflammatory response and decrease MMP activity in macrophages.

In contrast to its effect on outward remodeling, no inhibitory effect of L-Pred on IH in the venous outflow tract was observed. These results deviate from other preclinical studies that evaluated the therapeutic effect of GCs in other vascular injury models, that have reported a strong inhibitory effect of dexamethasone on IH[25-27]. This discrepancy may result from a difference in potency between prednisolone and dexamethasone to inhibit VSMC proliferation[28]. Alternatively, it may relate to the difference in pathophysiological stimuli that contribute to IH after arterial injury, when compared to venous IH in AVF. While hemodynamic stimuli are considered to be of vital importance for IH[29,30], the contribution of inflammation to IH in AVF might be limited, as suggested by our results. As a consequence, interventions that facilitate OR might therefore also result in a (modest) stimulation of IH. Ultimately, the net result of vascular remodeling in the AVF was increased luminal area of the venous outflow tract, that was mainly accountable by the OR process alone. Of note, a stimulatory effect of an intervention on OR is more important for the ultimate luminal surface area than the coinciding effect on IH, as there is a quadratic relationship between radius and surface area of the vessel.

In conclusion, liposomal prednisolone reduces the local inflammatory response and stimulates venous outward remodeling in murine AVF. Therefore, treatment with liposomal prednisolone might be valuable strategy to reduce AVF non-maturation. The efficacy of liposomal prednisolone to enhance radiocephalic AVF maturation in ESRD patients is currently being evaluated in the LIPMAT trial (clinicaltrial.gov ID NCT0249566), a double-blind, randomized, placebo-controlled trial[31].

Targeting outward remodeling—a new direction for drug development?

While previous studies focused mainly on the development of strategies that aimed to reduce intimal hyperplasia, the current view on AVF maturation underscores the link between impaired outward remodeling and AVF failure[8,32]. Investigation of new targets that may promote outward remodeling might be of a great interest.

One of the appealing candidates is hormone relaxin (RLN2), originally known for its role in the growth and differentiation of the reproductive tract, and systemic hemodynamic adaptations during pregnancy, in particular vasodilatation[33-35]. In the context of AVF maturation, it is important to emphasize that RLN2 is expressed in the vessel wall[36,37] and that the observed vascular effects of RLN2 are mediated though an interaction with RXFP1 (relaxin/insulin-like peptide family receptor 1, original abbreviation LGR7), a G-protein-coupled receptor. In **chapter 4** of this thesis we examined the consequences of disturbing this hormone-receptor balance in murine model of AVF failure. Our hypothesis was that RXFP1 deficiency disables local signal transduction from endogenous relaxin after AVF surgery resulting in reduced OR. Indeed, deficiency of RXFP1 resulted in a 22% decrease in vessel size at the venous outflow tract 14 days after AVF surgery.

One of the main physiological actions of relaxin is its ability to remodel extracellular matrix components such as collagen and elastin in the cervix and myometrium during pregnancy[38,39]. In the settings of AVF, MMPs expression must be augmented to degrade and restructure the vascular matrix[40] to promote outward remodeling. Interestingly, peri-adventitial application of recombinant elastase has been shown to stimulate outward remodeling in a rabbit-model of AVF[41]. In our study we observed a 43% increase in elastin content in the lesions of RXFP1 deficient mice which coincided with a 41% reduction in elastase activity, suggesting that RXFP1 is an important regulator of elastin degradation during AVF maturation. Furthermore, it supports the concept that venous outward remodeling requires relaxin axis-mediated augmentation of elastase activity.

Interestingly, relaxin-relaxin receptor interactions are known to mitigate vascular inflammation, by inhibiting the upregulation of pro-inflammatory cytokines[42]. In line with these observations, we found that RXFP1 deficiency augments vascular inflammation in AVFs, as illustrated by a 6-fold increase in $CD45^+$ leukocytes, along with a 2-fold increase in MCP1 expression in the venous outflow tract.

Despite elevated levels of MCP1 in AVF lesions of RXFP1 deficient mice, we unexpectedly did not observe effects on venous intimal hyperplasia. Further *in vitro* experiments revealed that RXFP1 ablation caused a phenotypic switch of both arterial and venous VSMCs from a contractile towards a synthetic phenotype, as illustrated by augmentation of collagen, fibronectin, TGFβ and PDGF mRNA expression levels. Functional studies revealed elevated migration of arterial and venous VSMCs isolated form RXFP1 deficient mice. The question arises why RXFP1 deficiency *in vivo* resulted in decreased outward remodeling and had no effect on intimal hyperplasia, whereas *in vitro* functional studies clearly show impact of RXFP1 deficiency on VSMCs migration. In this respect, it is important to notice that the efficacy of cell migration *in vivo* strongly depends on the balance between cell deformability and ECM density, of which the latter is governed by the capacity of proteolytic enzymes to degrade matrix components[43]. Interestingly, RXFP1 deficiency resulted in a significant increase in elastin content as a result of decreased elastase activity. This preserved elastin density most likely explains why the increased migratory capacity of RXFP1 deficient VSMCs *in vitro*, did not translate into enhanced intima hyperplasia in the venous outflow tract of AVF in RXFP1 deficient mice. Finally, RXFP1 and RLN expression levels were increased in human AVFs, as compared to unoperated cephalic veins, strongly suggesting that therapeutic targeting of this pathway in the context of AVF maturation could be beneficial.

In conclusion, RXFP1 deficiency hampers elastin degradation and results in induced vascular inflammation after AVF surgery. These processes impair outward remodeling in murine AVF, suggesting that the relaxin-axis could be a potential therapeutic target to promote AVF maturation.

Remaining questions, points of consideration

One of the puzzling observations coming across this thesis is the difference in anatomical origin, gene expression profile and functional behavior of arterial and venous VSMCs. As the VSMCs and myofibroblasts are the main cell types contributing to intimal hyperplasia formation, it is of vital importance to gain more insights on the source of these cells within the neointima.

In **chapter 5** of this thesis, the anatomical origin of VSMCs that are responsible for venous stenotic lesions in arteriovenous fistulas is discussed. In the past, migrated VSMCs from the venous tunica media were considered to be the most prominent source of neointimal cells, more recent studies suggest that venous adventitial fibroblasts, circulating vascular progenitor cells, and arterial VSMCs might contribute as well.

Liang and coworkers in an elegant lineage tracing study suggested that VSMCs from the anastomosed artery contributed to as much as 50% of the VSMC compartment in the venous intima. The underlying mechanism was increased Notch signaling—a pathway critical in vascular development, in particular determining arterial versus venous vessel formation.

Not only different origin of VSMCs, but their physiological state might steer the cellular response in AVF maturation, as recent study from Zhao *et al.* suggests. Genetic mapping displayed a dual function of mature VSMCs in AVF maturation, with differentiated/contractile VSMCs contributing to medial wall thickening towards beneficial venous maturation and dedifferentiated/proliferative VSMCs contributing to detrimental neointimal hyperplasia[44].

These findings underscore importance to further understand the role of different VSMCs in AVF maturation. While discussing implementation of the inhibitors of VSMCs proliferation, such as Notch/FSP-1, to improve AVF patency we should keep in mind that complete inhibition of VSMC proliferation and migration in the early phase after AVF surgery does not necessarily translate into a better functional outcome of AVFs. In previous chapters of this thesis we have already discussed importance of VSMC proliferation as a prerequisite for adequate outward remodeling of the involved blood vessels. Therefore, the timing of the application of novel interventions to inhibit VSMC proliferation could be crucial for its effect on the functional outcome of the AVF. Another attractive aim for the future studies is to optimize venous adaptation to the arterial environment during postsurgical processes.

Beyond arteriovenous fistula

Even though AVF is preferred modality of hemodialysis vascular access site, native veins are often unavailable due to preexisting vascular pathology[12]. Utilization of synthetic vascular grafts predominantly results from the development of intimal hyperplasia ultimately leading to graft occlusion, and a relatively high risk of infectious

complications[45,46]. To create tissue engineered vascular grafts is one of the promising solutions to overcome current limitations of synthetic grafts and diseased native blood vessels. Previously, we developed a method to generate autologous TEBVs *in vivo*, which is based on the FBR directed to a subcutaneously implanted polymer rod that culminates in the formation of a fibrocellular TC[47]. Thus far, the origin of the cells present within the TCs remains unknown. Understanding the origin of cells present in the TC is of vital importance for its application as vascular grafts, as various disease conditions such as diabetes mellitus and CKD coincide with impaired function of BM-derived cells[48,49], which could hamper TC formation.

In **chapter 6** of this thesis, we elucidated the contribution of BM-derived cells in TC formation. For this purpose, we implanted polymer rods in the subcutis of rats after receiving BM-transplants with GFP-labeled BM cells. In addition, a CKD model was incorporated, as we aim to utilize the engineered vascular grafts for patients with ESRD requiring vascular access for hemodialysis.

In the early phase after rod implantation, TCs were mainly composed of $CD68^+$ macrophages which where predominantly located along the inner border, adjacent to the polymeric rod. On average, 13% of $CD68^+$ macrophages were GFP^+ cells, indicating BM origin. Several studies suggest that cells within the local tissue environment, such as tissue-resident macrophages, also contribute to the foreign body response and play major role in the formation of engineered tissue[50,51]. Here, we show that the maturation of the TC is associated with a 40% increase in repair associated $CD68^+/CD163^+$ macrophages along with increase in IL10 and pro-fibrotic TGFβ mRNA levels within TCs.

During the development of the TC, we observed a gradual transition from granulation tissue towards circumferentially aligned SMA^+ myofibroblasts. Macrophage-to-myofibroblasts differentiation appeared to play an important role in TC formation as 26% of SMA^+/GFP^+ myofibroblasts co-expressed macrophage marker CD68. Interestingly, we detected a population of $CD133^+$ bone marrow progenitor cells, positive for GFP, from which 24% were positive for SMA^+ suggesting that $CD133^+$ BM-derived cells can also contribute to the myofibroblast population in mature TC.

Finally, we did not observe a significant effect of CKD on the cellular response upon implantation of the polymer rod. We hypothesize that the local foreign body response upon implantation of the polymer rod is substantially differs from chronic inflammation and subsequent tissue fibrosis as observed in various organs of patients with CKD. After only 3 weeks, tissue capsules were well matured, indicating the importance of the acute response and that the local environment within subcutaneous space is sufficient to maintain the process of TC formation.

Overall results from our study show that both BM-derived, as well as tissue resident cells, contribute to TC formation, whereas macrophages serve as precursors of myofibroblasts in mature TCs. The presence of CKD did not significantly alter the process of TC formation, which supports our approach for future clinical use in ESRD patients.

Final conclusion

Dysfunction of vascular access remains a major clinical problem responsible for high morbidity and substantial health care costs in patients required chronic hemodialysis treatment. Despite the magnitude of this clinical problem, there have been no major novel therapeutic interventions in the field of hemodialysis access for the past few decades.

Even though nowadays more and more attention is drawn onto importance to understand pathobiology of vascular access dysfunction the complete picture is still missing.
In our group, we developed a unique murine AVF model, which has a configuration similar to the one used most frequently in humans (venous end-to-arterial side). This work has revealed that stenotic lesions in this murine AVF model closely resemble that in human failed fistulas. Specifically, pathological lesions are localized near the venous anastomosis and are characterized by proliferating VSMCs, infiltration of leukocytes and accumulation of extracellular matrix components.

Work described in this thesis, further shed light onto our understanding of the pathophysiology of AVF failure and identified new therapeutic targets aimed to improve patency of AV-fistula.

We first explored the role of inflammation in vascular remodeling upon AVF surgery. We unraveled the complex role of natural agonist of TLR4–RP105 in the pathophysiology of AVF failure identifying a novel relationship between inflammation and VSMC function.

Knowing that AVF surgery triggers influx of inflammatory cells in the vessel wall, in our subsequent study we focused on the strategy to reduce inflammation by administering liposomal prednisolone. We observed reduction in the local inflammatory response and increase in the venous outward remodeling, suggesting that local delivery of prednisolone via liposomes is beneficial for AVF maturation.

Based on the evolving concept of the importance of the outward remodeling in AVF maturation next, we evaluated the role of relaxin pathway as potential contributor to OR in AVF maturation. Deficiency of the relaxin receptor resulted in an impaired outward remodeling in the murine AVF, suggesting that the relaxin-axis could be a potential therapeutic target to promote AVF maturation. Together with our collaborators at the university Miami, Florida in near future we aim to test therapeutic agonist of relaxin in our AVF model and in hemodialysis patients.

In parallel with testing new therapeutics to improve maturation and patency of native AVFs, our group is working on developing TEBVs which could offer a suitable alternative for arteriovenous conduits. In collaboration with University of California, Los Angeles, we performed a lineage tracing to study the contribution of bone marrow derived cells to TEBV formation and the impact of CKD onto the process of TEBV formation. Importantly, the CKD condition did not significantly alter the process of

TEBV formation, supporting our technology to be relevant for future clinical use in ESRD patients.

Some aspects of our studies require further discussion. One of the limitations in our experimental setup is the inability to perform flow measurements and cannulations of the murine AVF, as an adequate blood flow volume and the cannulability of the AVF are the main characteristics of functional hemodialysis access.

Another point to consider is that our studies were performed in healthy mice. Recently established CKD model described by Kang *et al.* demonstrated that fistula maturation is affected by CKD, specifically the chronic accumulation of waste products and uremic toxins in the blood impacted AVF flow, resulting in increased venous wall thickness and thrombus formation[52].

However, we believe that decision on combining AVF model with CKD, should be well-reasoned and based not only on blind faith, but ethical justification. Welfare of laboratory animals is an important parameter of consideration while designing a new study. In studies aimed to identify new pathways involved in the pathobiology of AVF, the relevance of CKD implementation should be questioned.

For many years, vascular access was regarded as an exclusively surgical problem. Up to now, advances in vascular access treatments are limited. Medical professionals often focusing on the one-center experience and neglect importance to study the fundamental mechanisms leading to an arteriovenous fistula failure. It is time to recognize vascular access as truly multi-disciplinary science and to bring fields of molecular biology, medicine and new advances of biomaterials for local drug delivery to improve current patient care.

Reference List

1. Kolff, W.J. The artificial kidney. *Journal of the Mount Sinai Hospital, New York* **14**, 71-79 (1947).
2. Cooley, D.A. In Memoriam: Willem Johan Kolff 1911–2009. *Texas Heart Institute Journal* **36**, 83-84 (2009).
3. Scribner, B.H., Buri, R., Caner, J.E., Hegstrom, R. & Burnell, J.M. The treatment of chronic uremia by means of intermittent hemodialysis: a preliminary report. *Transactions - American Society for Artificial Internal Organs* **6**, 114-122 (1960).
4. Cimino, J.E. & Brescia, M.J. Simple venipuncture for hemodialysis. *The New England journal of medicine* **267**, 608-609 (1962).
5. Brescia, M.J., Cimino, J.E., Appel, K. & Hurwich, B.J. Chronic hemodialysis using venipuncture and a surgically created arteriovenous fistula. *The New England journal of medicine* **275**, 1089-1092 (1966).
6. Renine.nl. RENINE-year-report. *Online Source* (2016).
7. Clinical practice guidelines for vascular access. *American journal of kidney diseases : the official journal of the National Kidney Foundation* **48 Suppl 1**, S248-273 (2006).
8. Lee, T. & Misra, S. New Insights into Dialysis Vascular Access: Molecular Targets in Arteriovenous Fistula and Arteriovenous Graft Failure and Their Potential to Improve Vascular Access Outcomes. *Clinical journal of the American Society of Nephrology : CJASN* **11**, 1504-1512 (2016).
9. Rothuizen, T.C., *et al.* Arteriovenous access failure: more than just intimal hyperplasia? *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* **28**, 1085-1092 (2013).
10. Wong, C.Y., *et al.* Vascular remodeling and intimal hyperplasia in a novel murine model of arteriovenous fistula failure. *Journal of vascular surgery* (2013).
11. Wang, Y., *et al.* Venous stenosis in a pig arteriovenous fistula model--anatomy, mechanisms and cellular phenotypes. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* **23**, 525-533 (2008).
12. Lee, T., *et al.* Comparative analysis of cellular phenotypes within the neointima from vein segments collected prior to vascular access surgery and stenotic arteriovenous dialysis accesses. *Seminars in dialysis* **27**, 303-309 (2014).
13. Lee, T. & Haq, N.U. New Developments in Our Understanding of Neointimal Hyperplasia. *Advances in chronic kidney disease* **22**, 431-437 (2015).
14. Nath, K.A., Kanakiriya, S.K., Grande, J.P., Croatt, A.J. & Katusic, Z.S. Increased venous proinflammatory gene expression and intimal hyperplasia in an aorto-caval fistula model in the rat. *The American journal of pathology* **162**, 2079-2090 (2003).
15. Hollestelle, S.C., *et al.* Toll-like receptor 4 is involved in outward arterial remodeling. *Circulation* **109**, 393-398 (2004).
16. Karper, J.C., *et al.* Blocking toll-like receptors 7 and 9 reduces postinterventional remodeling via reduced macrophage activation, foam cell formation, and migration. *Arteriosclerosis, thrombosis, and vascular biology* **32**, e72-80 (2012).
17. Vink, A. In Vivo Evidence for a Role of Toll-Like Receptor 4 in the Development of Intimal Lesions. *Circulation* **106**, 1985-1990 (2002).
18. Liang, M., *et al.* Migration of smooth muscle cells from the arterial anastomosis of arteriovenous fistulas requires Notch activation to form neointima. *Kidney international* **88**, 490-502 (2015).
19. Skartsis, N., *et al.* Origin of neointimal cells in arteriovenous fistulae: bone marrow, artery, or the vein itself? *Seminars in dialysis* **24**, 242-248 (2011).

20. Cronstein, B.N., Kimmel, S.C., Levin, R.I., Martiniuk, F. & Weissmann, G. A mechanism for the antiinflammatory effects of corticosteroids: the glucocorticoid receptor regulates leukocyte adhesion to endothelial cells and expression of endothelial-leukocyte adhesion molecule 1 and intercellular adhesion molecule 1. *Proceedings of the National Academy of Sciences of the United States of America* **89**, 9991-9995 (1992).
21. Rhen, T. & Cidlowski, J.A. Antiinflammatory action of glucocorticoids--new mechanisms for old drugs. *The New England journal of medicine* **353**, 1711-1723 (2005).
22. Oray, M., Abu Samra, K., Ebrahimiadib, N., Meese, H. & Foster, C.S. Long-term side effects of glucocorticoids. *Expert opinion on drug safety* **15**, 457-465 (2016).
23. Galis, Z.S. & Khatri, J.J. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. *Circulation research* **90**, 251-262 (2002).
24. Nieves Torres, E.C.*, et al.* Adventitial Delivery of Lentivirus-shRNA-ADAMTS-1 Reduces Venous Stenosis Formation in Arteriovenous Fistula. *PloS one* **9**, e94510 (2014).
25. Pires, N.M.*, et al.* Histopathologic alterations following local delivery of dexamethasone to inhibit restenosis in murine arteries. *Cardiovascular research* **68**, 415-424 (2005).
26. Schepers, A.*, et al.* Short-term dexamethasone treatment inhibits vein graft thickening in hypercholesterolemic ApoE3Leiden transgenic mice. *Journal of vascular surgery* **43**, 809-815 (2006).
27. Villa, A.E.*, et al.* Local delivery of dexamethasone for prevention of neointimal proliferation in a rat model of balloon angioplasty. *The Journal of clinical investigation* **93**, 1243-1249 (1994).
28. Reil, T.D., Sarkar, R., Kashyap, V.S., Sarkar, M. & Gelabert, H.A. Dexamethasone suppresses vascular smooth muscle cell proliferation. *The Journal of surgical research* **85**, 109-114 (1999).
29. Roy-Chaudhury, P., Spergel, L.M., Besarab, A., Asif, A. & Ravani, P. Biology of arteriovenous fistula failure. *Journal of nephrology* **20**, 150-163 (2007).
30. Asif, A., Roy-Chaudhury, P. & Beathard, G.A. Early arteriovenous fistula failure: a logical proposal for when and how to intervene. *Clinical journal of the American Society of Nephrology : CJASN* **1**, 332-339 (2006).
31. Voorzaat, B.M.*, et al.* Improvement of radiocephalic fistula maturation: rationale and design of the Liposomal Prednisolone to Improve Hemodialysis Fistula Maturation (LIPMAT) study - a randomized controlled trial. *The journal of vascular access* **18**, 114-117 (2017).
32. Guzman, R.J., Abe, K. & Zarins, C.K. Flow-induced arterial enlargement is inhibited by suppression of nitric oxide synthase activity in vivo. *Surgery* **122**, 273-279; discussion 279-280 (1997).
33. Conrad, K.P., Debrah, D.O., Novak, J., Danielson, L.A. & Shroff, S.G. Relaxin modifies systemic arterial resistance and compliance in conscious, nonpregnant rats. *Endocrinology* **145**, 3289-3296 (2004).
34. Feng, S., Bogatcheva, N.V., Kamat, A.A., Truong, A. & Agoulnik, A.I. Endocrine effects of relaxin overexpression in mice. *Endocrinology* **147**, 407-414 (2006).
35. Jeyabalan, A., Shroff, S.G., Novak, J. & Conrad, K.P. The vascular actions of relaxin. *Advances in experimental medicine and biology* **612**, 65-87 (2007).
36. Novak, J.*, et al.* Evidence for local relaxin ligand-receptor expression and function in arteries. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **20**, 2352-2362 (2006).
37. Jelinic, M.*, et al.* Localization of relaxin receptors in arteries and veins, and region-specific increases in compliance and bradykinin-mediated relaxation after in vivo serelaxin treatment. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **28**, 275-287 (2014).

38. Finlay, G.A., O'Donnell, M.D., O'Connor, C.M., Hayes, J.P. & FitzGerald, M.X. Elastin and collagen remodeling in emphysema. A scanning electron microscopy study. *The American journal of pathology* **149**, 1405-1415 (1996).
39. Chen, B., Wen, Y., Yu, X. & Polan, M.L. Elastin metabolism in pelvic tissues: is it modulated by reproductive hormones? *American journal of obstetrics and gynecology* **192**, 1605-1613 (2005).
40. Chan, C.Y., Chen, Y.S., Ma, M.C. & Chen, C.F. Remodeling of experimental arteriovenous fistula with increased matrix metalloproteinase expression in rats. *Journal of vascular surgery* **45**, 804-811 (2007).
41. Peden, E.K.*, et al.* Arteriovenous fistula patency in the 3 years following vonapanitase and placebo treatment. *Journal of vascular surgery* **65**, 1113-1120 (2017).
42. Brecht, A., Bartsch, C., Baumann, G., Stangl, K. & Dschietzig, T. Relaxin inhibits early steps in vascular inflammation. *Regulatory peptides* **166**, 76-82 (2011).
43. Wolf, K.*, et al.* Physical limits of cell migration: control by ECM space and nuclear deformation and tuning by proteolysis and traction force. *The Journal of cell biology* **201**, 1069-1084 (2013).
44. Zhao, J.*, et al.* Dual Function for Mature Vascular Smooth Muscle Cells During Arteriovenous Fistula Remodeling. *Journal of the American Heart Association* **6**(2017).
45. Rotmans, J.I.*, et al.* Hemodialysis access graft failure: time to revisit an unmet clinical need? *Journal of nephrology* **18**, 9-20 (2005).
46. Roy-Chaudhury, P.*, et al.* Venous neointimal hyperplasia in polytetrafluoroethylene dialysis grafts. *Kidney international* **59**, 2325-2334 (2001).
47. Rothuizen, T.C., et al. Development and evaluation of in vivo tissue engineered blood vessels in a porcine model. *Biomaterials* 75, 82-90 (2016).
48. Westerweel, P.E.*, et al.* Impaired endothelial progenitor cell mobilization and dysfunctional bone marrow stroma in diabetes mellitus. *PloS one* **8**, e60357 (2013).
49. Kato, S.*, et al.* Aspects of immune dysfunction in end-stage renal disease. *Clinical journal of the American Society of Nephrology : CJASN* **3**, 1526-1533 (2008).
50. Okabe, Y. & Medzhitov, R. Tissue biology perspective on macrophages. *Nature immunology* **17**, 9-17 (2015).
51. Cailhier, J.F.*, et al.* Conditional macrophage ablation demonstrates that resident macrophages initiate acute peritoneal inflammation. *Journal of immunology (Baltimore, Md. : 1950)* **174**, 2336-2342 (2005).
52. Kang, L.*, et al.* A new model of an arteriovenous fistula in chronic kidney disease in the mouse: beneficial effects of upregulated heme oxygenase-1. *American journal of physiology. Renal physiology* **310**, F466-476 (2016).

Chapter 8

Nederlandse Samenvatting

Curriculum Vitae

List of Publications

Acknowledgement