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## Unraveling mechanisms of vascular remodeling in arteriovenous fistulas for hemodialysis

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### Citation

Wong, C. Y. (2017, March 8). *Unraveling mechanisms of vascular remodeling in arteriovenous fistulas for hemodialysis*. Retrieved from <https://hdl.handle.net/1887/46406>

Version: Not Applicable (or Unknown)

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**Issue Date:** 2017-03-08

# Chapter 1

General Introduction





## End stage renal disease

The kidney is an intricate organ that has many life-sustaining functions, such as removing waste products and excess fluid from the body through secretion of urine, maintaining the balance/homeostasis of electrolytes, acidity and blood pressure. Kidney disease can ultimately lead to permanent failure of the kidney function. The most frequent causes for kidney failure are diabetes mellitus and hypertension. When the kidney function worsens to the stage that the patient is life dependant on renal replacement therapy, the patient then suffers from end-stage renal disease (ESRD). The number of ESRD patients in the Netherlands on January 2015 was 16.316 and every year there are about 2000 new ESRD patients<sup>1</sup>.

The treatment for ESRD consists of kidney replacement therapy, which can be divided in two modalities: Kidney transplantation and dialysis. The transplantation of a donor kidney is the preferred type, and although the number of patients with functioning donor kidneys is growing in the Netherlands, there is still a large group of patients who do not have a donor kidney mainly due to the unavailability of donor organs. This group of patients is dependent on dialysis treatment.

## Dialysis treatment

There are two dialysis treatment modalities: Haemodialysis (HD) and peritoneal dialysis (PD). In haemodialysis treatment, the exchange of substances and extraction of fluids are carried out directly via the blood, whereas peritoneal dialysis is carried out via the peritoneal cavity. Haemodialysis treatment generally requires patients to travel to the hospital for about 3 times a week. Despite the latter, HD treatment (86%) is favoured greatly over PD (14%) in the Netherlands. On a world-wide scale, the number of haemodialysis patients adds up to approximately 2 million.

## Vascular access

One of the prerequisite of HD treatment is gaining access to a blood vessel with a high blood flow. The most simple and quickest option is to gain access to the central venous system using a catheter. However, this direct form of vascular access is not preferred for chronic dialysis due to the high risk of catheter related infections and central vein stenosis. A better and a more practical location for the vascular access point would be in the upper extremity. The veins in the lower arm are located superficially and are therefore suited for easy percutaneous cannulation. However, these veins lack the required high blood flow capacity that is needed for HD. Fortunately, a high blood flow can be acquired surgically by connecting the high pressure arterial system with the low pressure venous system. A direct connection between the artery and vein is called an arteriovenous fistula (AVF) and when a prosthetic graft is used it is called

an arteriovenous graft (AVG). The National Kidney Foundation Dialysis Outcomes Quality Initiative (NKF KDOQI) advocates the use of AVFs whenever possible. This is due to a lower rate of mortality, infection and a higher patency of the AVF when compared to the AVG<sup>2,3</sup>. However, a disadvantage of the AVF is that it needs to “mature” before it can be used. This is a complex process in which the diameters and the blood flow of the involved vessels increase in order to facilitate HD. According to the KDOQI an AVF is matured when the fistula has a flow of at least 600 ml/min, a venous diameter of at least 6 mm and is located no deeper than 6 mm under the skin. The focus of this thesis will be primarily on the AVF.

### **Pathophysiology of vascular access failure (chapter 1)**

Although the AVF is the preferred form of vascular access for chronic haemodialysis, it has significant problems with vascular access dysfunction responsible for a high morbidity rate<sup>4-6</sup>. Moreover, vascular access-related problems account for more than 25% of all hospitalizations in ESRD patients<sup>4</sup> surmounting to more than 1 billion dollars per year being spent on access-related care in the US alone.

From a time point field of view, AVF failure can be categorized in early- and late failure, in which early failure has been defined as an AVF that has never developed adequately for dialysis (maturation failure), or which fails within 3 months of starting dialysis treatment. All other failures are categorized as late failures.

One of the key parameter that determines the success of an AVF is the amount of blood flow that the fistula can facilitate, which is mainly dependant on the available luminal area. How the luminal area develops after AVF creation is mainly determined by two morphological processes that can balance each other out: [1] Intimal hyperplasia (IH) and [2] Outward remodeling (OR). Intimal hyperplasia is the classical histological lesion that appears to be associated with AVF failure, and is mainly composed of vascular smooth muscle cells (VSMCs)<sup>7</sup>. Importantly, IH is considered to be an unwanted process that needs to be reduced as much as possible. On the other hand, outward remodeling is defined by the increase in the vascular diameter and is considered to be a favourable process that can potentially increase the luminal area. The end result that determines the luminal area, and ultimately blood flow, is dependent on the two processes. Which factors determine the favourable or unfavourable outcome remains unclear. In the current literature, therapeutic interventions are predominantly aimed at the reduction of intimal hyperplasia, thereby potentially neglecting the beneficial effects of stimulating outward remodeling.

It is clear that AVF failure remains a significant clinical problem that needs to be improved. In order to develop new therapeutic strategies, it is of utmost importance to first unravel the pathophysiology that is responsible for AVF failure.

## Animal models

Although it is still unclear what mechanisms exactly drive maturation failure, the pathogenesis of IH has been well described in the literature and seems to be multifactorial. Factors that are believed to contribute to IH are: (1) Surgical trauma, (2) unfavourable hemodynamic shear stress<sup>8,9</sup>, (3) vessel injury due to dialysis needle, (5) uraemia resulting in endothelial dysfunction<sup>10</sup> and (6) chronic inflammatory state associated with ESRD<sup>11,12</sup>.

Due to the complex nature of the multiple processes involved in AVF failure from which the hemodynamical aspects seems to be of great influence, we believe that basic research is best to be performed in an in-vivo animal model. In the literature, numerous animal models in various animal species have been described that are used to study AVF failure, ranging from a baboon to the mouse<sup>13</sup>. In this great arsenal of animal models, we would prefer the murine variant, due to the widespread availability of genetically altered animals. This allows us to study gene specific factors that might contribute to AVF failure. There are multiple murine models available that all utilize the vasculature in the neck (common carotid artery and jugular vein), however both the models of Kwei et al.<sup>14</sup> and Yang et al.<sup>15</sup> describe a functional end-to-end AVF configuration using a synthetic cuff to complete the anastomosis. Castier et al.<sup>16</sup> introduced an improved murine AVF model in which the end of the common carotid artery was connected to the side of the ipsilateral jugular vein using micro sutures, thereby taking out the potentially pro-thrombotic and pro-inflammatory effect of the synthetic cuff of the equation. Although this new model is considered to be an important improvement, we believe that the AVF configuration can further be optimized. Unlike in the model described by Castier et al.<sup>16</sup>, AVFs in the clinical setting most frequently are constructed in a venous end to arterial side manner. This different configuration is very important, as this directly leads to a different hemodynamic profile in the AVF, which is an important factor in AVF biology. We therefore set out to develop a new murine AVF model that incorporates the same vascular configuration as is utilized in humans.

## Aims of thesis and thesis outline

The main aim of this thesis was to develop a novel murine model of AVF failure that can be used to further unravel the pathological factors that are involved in AVF maturation failure, in order to develop a basis for the development of novel therapies. Although the exact mechanisms underlying AVF failure remains unclear, both IH and inadequate outward remodelling seem to contribute.

In **chapter 2** the possible factors that might contribute to AVF failure are discussed.

In **chapter 3 and 4** we describe our newly developed murine model of AVF failure, which incorporates a similar vascular configuration as is utilized in humans. We also used an unconventional technique (near infrared fluoroscopy) to visually assess the patency of the AVF in our murine model.

In **chapter 5** we assessed the role of the extracellular matrix protein elastin in vascular remodelling using a mouse that was haplodeficient for the tropoelastin gene. Elastin is the principle extracellular matrix (ECM) protein in the arterial wall constituting about 30-50% of the dry weight of arteries<sup>17</sup>. It provides the blood vessel its unique mechanical property that is needed for proper function. Previous studies have shown that elastin is the major inhibitor of VSMC migration and proliferation<sup>18</sup>. Interestingly, the basal elastin content in veins is relatively low compared to arteries. The latter suggest that the enhanced proliferative response in vein grafts (when compared to arterial grafts) may relate to the less pronounced protective function of elastin in veins. Surprisingly, the role of elastin in AVF failure has not received much attention thus far.

Previous studies have shown that the inflammatory response is involved in the development of IH in vascular injury<sup>19-21</sup>. However, the role of inflammation in AVF biology has not yet been elucidated. Nonetheless, there are clues that AVF failure is linked to inflammation. In a study by Liu et al., higher levels of inflammatory blood markers such as high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- $\alpha$ ) was associated with AVF dysfunction in haemodialysis patients. In **chapter 6**, we investigate the role of inflammation in AVF failure by using prednisolone in our murine model. To improve local biological activity, we encapsulated prednisolone in liposomes acting as a local target delivery vehicle.

In **chapter 7** we study the effects of the TLR4 inhibitor RP105 on AVF remodeling. RP105 is a structural homologue of TLR4 that lacks the internal signaling domain and can act as an inhibitor of the TLR4 mediated signaling. Based on the hypothesis that TLR4 mediated inflammatory responses contribute to vascular remodeling in several pathophysiological processes, we anticipate an increased AVF maturation in mice that are deficient of the TLR4 inhibitor RP105. Therefore we performed AVF surgery in RP105 deficient mice and studied the effects on the AVF maturation in vivo. In addition we studied in a series of in vitro experiments the effects of RP105 deficiency on smooth muscle cells and inflammatory cells in more detail.

**Chapter 8** summarizes the findings in this thesis and reviews future perspectives in the field of AVF research.



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