Experimental studies on hemodialysis access innovations

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A proof-of-principle
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Wouter Jan Geelhoed, MSc,[1,2] Resh
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Wouter J. Geelhoed[1,2] · Loren

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2. Introduction

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Chapter 7

Summary and discussion

Summary

Worldwide, the incidence of chronic kidney disease (CKD) and end stage renal kidney (ESKD) is increasing each year, and with it the number of patients reliant on dialysis for survival[1]. The most common form of dialysis is hemodialysis (HD), where the blood is filtered by an extracorporeal dialysis machine. Without a suitable vascular access (VA) site, an efficient HD procedure cannot be performed. Therefore, an arteriovenous fistula (AVF) or arteriovenous graft (AVG) is used to create long-term VA sites. While AVF are prone to maturation failure, AVG are prone to infection, while both are prone to the formation of stenotic lesions [2-6]. An alternative to AVF and AVG are tissue engineered blood vessels (TEBVs)[7]. One method of producing TEBVs by utilizing the foreign body response (FBR) to biomaterials to generate *in vivo* TEBVs has been discussed in this thesis (**Chapter 2**). The subcutaneous implantation of a foreign body results in the formation of a fibrous tissue capsule (TC). This TC can then be grafted in the vasculature to form a TEBVs.

One of the most vital aspects of TEBVs is their ability to withstand the mechanical forces induced by arterial blood pressure, thus ensuring patient safety[7,8]. In **chapter 3**, we compare three methods of evaluating the mechanical strength of biological vessels and discuss their suitability for use with TEBVs. We show that the derived methods known as the circumferential tensile strength and probe burst pressure methods provide an overestimation of the pressurized burst pressure in both biological and synthetic material. Moreover, the outcomes of the three test modalities evaluating the mechanical properties of different types of biological blood vessels correlate poorly with each other. Indirect methods of burst pressure assessment should thus not be used for the evaluation of TEBVs unless perfect homogeneity of the sample is assumed, and a high correlation is validated.

In **chapter 4**, we evaluate the remodeling capacity of autologous *in vivo* TEBVs for arteriovenous grafting in a large animal model. Histology showed that the initially implanted TCs were primarily composed of fibroblasts, myofibroblasts, and collagen. Within a month of vascular grafting, the TCs showed a notable increase in desmin-positive VSMC-like cells, as well as a notable decrease in macrophages. Two months after grafting, the number of myosin-HC–positive cells had increased further, and a clearly defined endothelial lining was present. One of the main experiments of this study were the functionality tests of the endothelial monolayer of the TEBVs. A custom-built *ex vivo* microfluidic perfusion chamber was specially designed to house large vascular tissue sections that could subsequently be perfused in parallel with blood drawn directly from a living animal. The system ensured that all samples were exposed to identical volumes of blood and shear stress levels. TEBV samples were shown to have less adherent blood components than TC samples. This indicated that the initial lack of endothelial cell coverage of the TCs does not result in acute thrombosis and that the observed endothelial monolayer of the TEBVs that developed in the 8 weeks after grafting was indeed non-thrombogenic *in vivo*. The burst pressure and suture retention strengths of the TEBVs exceeded the requirements for safe grafting, which are generally defined by the mechanical properties of the human saphenous vein [8,9]. A contractility assay revealed an increase in contractility of TEBVs at one-month post grafting compared to TC. This coincides with the relative increase in histological contractility markers in the TEBVs. One month after vascular grafting, 4 of the 5 TEBV grafts and 5 of the 6 ePTFE grafts remained

patent; two months after grafting, 2 out of 3 TEBV grafts and 1 out of 3 ePTFE grafts were still patent. At 1 month, stenosis in the non-occluded (i.e., patent) TEBV grafts was higher than in the non-occluded ePTFE grafts (52.22 ± 29.10% versus 4.17 ± 1.95%, respectively); in contrast, at 2 months stenosis in the TEBV grafts was lower than in the ePTFE grafts (12.94 ± 16.19% versus 85.61%, respectively).This study underlines the remodeling capacity of autologous TEBV *in vivo.* Venous stenosis however remains a problem for both ePTFE and TEBV grafts, a limitation which will need to be monitored during the clinical implementation of autologous TEBV.

Important in the clinical implementation of subcutaneously grown autologous *in vivo* TEBVs is the development of a non-invasive method to quantify TEBV maturation. Following implantation of the TC a layer of macrophages forms around the implant that drives the FBR. As the number macrophages decrease, the collagen content of the TC is known to increase. In **Chapter 5** we evaluate a novel folate-receptor-α (FR-α) agonist conjugated with the NIR fluorophore Indocyanine green (ICG) in order to quantify these macrophages over time. A decreased NIR signal was expected in accordance with a decrease in the number of macrophages. Contrary to the hypothesis, the NIR imaging revealed an increasing trend of fluorescence levels over time. Moreover, no NIR signal was observed to originate from the TC or from TC macrophages. Further analysis revealed the NIR signal originated exclusively from the skin layer above the TC. As the signal did not directly reflect TC processes it was concluded that this fluorophore was not an ideal candidate to monitor TC maturation. The FBR to biomaterials intrinsically aims to isolate an implant from the body through its encapsulation in a fibrous capsule. Due to its low circulation half-life the fluorophore likely did not reach the isolated TC macrophages. The fluorophore did however show potential for the non-invasive transcutaneous quantification of folate.

A common adverse event of this blood vessel cannulation is miscannulation with infiltration of the second part of the vessel wall, often resulting in a hematoma [10]. Although hematoma formation is always a burden for patients, its effects on ESKD patients requiring hemodialysis can be particularly detrimental due to the high blood flow of the VA [11]. Current dialysis needles types do not allow automated retraction of a needle in response to blood vessel cannulation and rely on manual user retraction. **Chapter 6** describes the design and proof-of-principle assessment of a novel needle design that rapidly automatically retracts in response to fluid contact. The system is based on the loss of tensile strength of a cellulose membrane upon fluid contact. The system was successfully optimized and showed automated retraction within 40ms upon cannulating a vessel pressurized to 100mmHg. As this study was a proof-of-principle assessment of the concept, further work is needed. Prior to this needle system being introduced in the clinic it must be incorporated into either an existing needle system or designed to comply with medical device regulations. Further benchtop assessment of the system should be carried out to further optimize the needle design.

Discussion and future perspectives

VA is essential for the survival of HD patients, where the gold standard intervention is the AVF [12]. Yet, due to its numerous downsides the AVF can also be viewed as a surgically induced

pathological condition. The placement of an AVF increases the risk of heart failure, damages blood vessels resulting in poor patency rates. In addition, AVF cannulation is painful and aesthetically unpleasant due to aneurysm formation, thereby causing a substantial burden for patients [12-14]. It may therefore be viewed as a necessary evil for HD patients.

The need for innovation in field of HD and VA is clear. The incidence of ESKD is increasing and will result in more patients requiring HD and a VA [1]. The healthcare costs associated with VA totaled to a staggering $3 billion in 2016 in the US, a number expected to be a third higher if private care costs are included [15].Various innovations attempting to improve VA outcomes are in development. Examples are the endoAVF systems, where a catheter-based device uses heat and pressure to create an AVF [16]. The endoAVF system is however limited to certain vascular anatomies. Fist Assist devices aim to improve AVF outcomes through venous dilation prior to AVF surgery, and although training of the forearm prior to AVF surgery is promising, data supporting these devices themselves are currently insufficient[17-19]. The improvement of AVF maturation and patency through pharmacological intervention is also under development[20]. Although as AVF pathology is immensely complex and not entirely understood, therapeutic intervention is complicated. Evidence of this may be the outcome of the phase 3 PATENCY-2 clinical study which failed to meet its primary endpoint. In this trial a recombinant elastase was administered to AVF during surgery, in the hope to stimulate outward remodeling [21]. An incomplete understanding of the exact pathophysiology of AVF failure may have, among other factors, contributed to the failure of this investigational compound. VA for HD is also an interesting field for vascular tissue engineering (VTE) [7]. VA for HD provides a stringent test case for any TEBV technology due to the high failure rate of both AVF and AVG. A long patency of a TEBV as VA will likely translate to a long patency as other vascular indications such as arterial bypass interventions. The risk to patients is relatively low, as the occlusion of a TEBV as arteriovenous conduit will restore normal arterial and venous flow. In contrast, the failure and occlusion of a TEBV as arterial bypass would result in loss of arterial blood flow and the risk of peripheral ischemia, making this application less suitable for a first-in-man study using TEBVs. In the field of VTE, the company Humacyte is currently conducting a phase 3 clinical trial of acellular TEBVs as arteriovenous grafts[22]. The success or failure of their technology may have large implications for the field of VTE. Data from their phase 2 study showed promising results although the 28% primary patency of the TEBV at 12 months was not superior to conventional ePTFE grafts [23].

The field of VTE itself is relatively new, which is evident by the lack of clear regulations specific for TEBVs regarding their functional assessment. Arguably the most vital characteristic of a TEBV is its ability to be safely grafted into the vasculature of a patient without mechanical failure (rupture due to pressure, suture rupture, aneurysm formation) [7,8]. Yet no consensus has been reached on what method for the mechanical assessment of TEBVs is optimal. The ISO7198 for the assessment of prosthetic grafts is often used as a starting point[24]. This document was however made for synthetic grafts and does not account for the variation that naturally occurs in biosocial tissues and vessels. The mechanical assessment of TEBVs has been performed differently in numerous studies[8,25-29]. Researchers often use derived methods of the gold standard pressurized burst pressure test, such as the circumferential tensile strength and probe burst pressure, to estimate TEBV burst pressure. It is tempting

to use a derived method, as these require smaller samples, while long segments of TEBVs needed for pressurized tests are often valuable samples. Moreover, studies frequently lack a detailed methodology, making it hard to deduce how the test was precisely performed. As the derived methods are based on a theoretical estimation of the real burst pressure, and do not consider biological variation, based on **chapter 3** of this thesis it is likely to assume that derived burst pressure estimations are often overestimated in literature.

Chapter 4 of this thesis describes the evaluation of autologous *in vivo* TEBV. The main competitor for these autologous TEBV are AVGs, as autologous vessels remain the ideal tissue for a vascular procedure. The TEBV displayed a cellular remodeling capacity towards a vascular phenotype through histology and contractility and attained a functionally non-thrombogenic endothelial monolayer. Moreover, as this was autologous tissue no rejection reaction is observed. AVG grafts on the other hand are available instantly and off-the-shelf, while these TEBVs require at least a month of subcutaneous maturation, excluding them from use in an acute setting. AVG are available in any required length, while the TEBVs are restricted to segments of max ~20cm.

Prior to the clinical application of the TEBV numerous steps must also be taken. The risk of aneurysm formation and vessel rupture must be taken extremely seriously, and prior to the clinical grafting of an *in vivo* TEBV several issues must be tackled. The manufacturing method of the implants must be standardized, and implants using a new production method must be validated *in vivo* in a large animal model. Changes in both the manufacturing method and polymer composition may have an unfavorable impact on the mechanical qualities of the implants and thus the composition of the formed TC. Therefore, TCs derived from polymeric implants intended for clinical use should be assessed for their pressurized burst pressure, suture retention strength, and long-term grafting safety if any change in the manufacturing method occurs. Changes in the manufacturing method may include a new manufacturing location, variations in the polymer composition or supplier, or other deviations from previously implemented productions protocols. The production method of the polymer implants presented in this thesis are reliant on chloroform-based surface modifications. These surface modifications are required to improve TC formation[30]. Chloroform exposure is associated with numerous health risks [31]. Solvent based etching of a polymer may lead to swelling of the polymer matrix, thus allowing the solvent to penetrate into the polymer [32]. Washing of the polymer implant following chloroform etching may remove the majority of surface chloroform, yet chloroform may be present within the polymer matrix itself. Post-implantation degradation or cracking of the polymer implant could therefore release chloroform into the body. The polymer implants should therefore be assessed for any chloroform residue both on the surface and within the polymer matrix structure prior to any clinical implantation. This may for example be done using nuclear magnetic resonance assessment utilizing the correct polymer solvents. Moreover, a study must be performed to assess the mechanical properties of the clinical implant as the subcutaneously implanted polymer must be able to withstand both the mechanical forces acting on it, such as bending of nearby tissue, and a potential external force of impact.

If the above requirements are met an initial clinical may be initiated to assess the mechanical properties of the *in vivo* TEBVs in humans. This clinical trial would not involve a vascular

grafting operation and only assess the growth of the subcutaneous TCS. It is vital that the mechanical properties of the TCs are extensively characterized in humans. Translation of animal study data to a clinical setting is notoriously unpredictable [33]. Data from the large animal study presented in this thesis should thus by no means be extrapolated to estimate the mechanical properties of similar TCs in humans. The mechanical quality of the TCs must be clinically validated using at least the SRS and pressurized burst pressure, as derived methods result in an overestimation of the results and should in never be used for the validation of biological tissue for a clinical study. Moreover, depending on the clinical application, a suitable trial population should be selected. For arteriovenous vascular access grafting a CKD patient population should be used in an initial trial. Although animal studies have indicated that CKD does not impact TC maturation [34], this must be confirmed in patients. The trial population should be sufficient to take into account patient variation with regard to TC maturation. This variation may arise due to CKD severity, or comorbidities such as diabetes or obesity. It is crucial for the *in vivo* TEBV method that optimal mechanical properties are attained upon vascular grafting. Although a method to non-invasively quantify TC maturation *in vivo* was attempted in **chapter 5** of this thesis, no applicable method currently exists. Prior to the initiation of a clinical trial involving TEBV grafting, a method should be developed and validated to accurately monitor TEBV maturation with regard to its mechanical properties. Such a method may be assessed in a first clinical evaluation of the TCs. Data from the first clinical trial should report positively report on the mechanical properties of the polymer implant, provide sufficient data regarding the variation within the intended patient population, and validate the non-invasive quantification of TC maturation. Subsequently, a clinical trial may be initiated to evaluate the safety and efficacy of *in vivo* TEBV as arteriovenous vascular access grafts. Aneurysm formation, anastomotic rupture, graft infection/inflammation should be closely monitored, and graft patency should be compared to that of AVF as standard of care.

Besides the problems associated with the patency of long term VA options, miscannulation during dialysis procedures has shown to be an underreported yet pressing issue[10,35]. Currently, there is no literature available that accurately describes the incidence of miscannulation events in dialysis patients in the Netherlands. In an effort to improve upon current VA cannulation procedures, proof-of-principle studies of a novel automated needle design has been outlined in this thesis (**chapter 6**). This validated the proposed concept as the base mechanism functioned reliably and well. The use of such a needle system may decrease the risk of miscannulation of VA sites, notably for new and less experienced personnel. Although, training and guidance of dialysis personnel, as well as ultrasound guided cannulation may be enough to significantly reduce the risk of miscannulation.

The increase in incidence of CKD and ESKD may in part be attributed to a sedentary lifestyle [1,36]. The prevention of a pathology is desirable over its incidence and treatment. Besides the focus on the downstream complications of CKD such as HD VA, a societal emphasis should be placed on promoting an active lifestyle in combination with dietary considerations such as a low sodium diet.

One way to improve VA for HD may be through the implementation of an adjustable anastomosis. Most VA associated complications arise through the change in vascular

hemodynamics. AVF and AVG placement results in an increased cardiac output, increasing the cardiac burden and risk of cardiac events[13]. Turbulent hemodynamics and high flow at the anastomosis have been associated with shunt stenosis, and AVF and AVG can both lead to VA steal syndrome[14,37]. Thus, ideally the anastomosis itself may be adjustable to allow for control of shunt flow. A thin synthetic graft section may be connected to the artery and vein in a side-to-side manner, thus normalizing blood flow when the graft is shut in a vertical manner. Such a system would allow the formation of an arteriovenous conduit only during dialysis procedures, potentially decreasing complications associated with permanent high arteriovenous flow.

To conclude, there is a clear societal need to improve upon current VA options, and numerous innovative paths are being explored by the field. Owing to the verified cellular remodeling capacity of TEBVs, the field of VTE may offer promising new VA options. To truly innovate VA for HD now and in the future, a multidisciplinary approach is essential to bringing about innovation, and progressing those innovations from mere ideas, to lifesaving treatments.

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