

SPECIFIC AIMS

Nearly 4 million people with end-stage renal disease in the United States require hemodialysis for renal replacement therapy with almost 65% of them using an arteriovenous fistula (AVF) for dialysis access (1). Unfortunately, approximately 30% of patients with AVFs require intervention within 6 months of starting hemodialysis and an additional 40-60% experience post-angioplasty restenosis within 1 year after the primary intervention, affecting about 2.3 million AVF hemodialysis patients. The main cause of loss of patency in AVFs is venous neointimal hyperplasia (VNH), the accumulation of vascular smooth muscle cells (VSMCs) in the vessel intima. Paclitaxel-coated balloons (PCBs) have shown overwhelming success in treating neointimal hyperplasia in coronary and peripheral arterial disease, which has driven several clinical trials to explore the use of PCBs in treating VNH in AVFs. Thus far, these results have been inconsistent and sometimes conflicting (2).

PCBs are designed to deposit crystalline paclitaxel on the vessel lumen when the balloon is deployed, after which the drug diffuses into the tissue to inhibit cell proliferation. This is one limitation of this treatment method: since PCBs rely on coating adhesion to the intima and diffusion of the drug into the tissue, flow-induced wall shear stress can cause drug loss by dislodging adhered particles. Another limitation of PCBs is the non-specificity of the drug. Paclitaxel targets any proliferating cell, so in addition to inhibiting VSMCs, it can inhibit endothelial cell proliferation and prevent reendothelialization. It can also cause off-target toxicity when coating particles are lost to systemic circulation. Therefore, better treatments for AVF stenosis are needed.

We propose a safer therapeutic drug and an alternative delivery method (**Figure 1**). RNA aptamers are “smart drugs” that have binding affinities and specificities comparable to antigen-antibody interactions. Aptamer 14 (Apt14) is one that selectively targets VSMCs and inhibits migration by blocking platelet-derived growth factor receptor- β (PDGFR- β) signaling (3). It has been found to reduce neointimal formation in murine (3) and porcine arterial injury models (4). We have also shown that Apt14 has no impact on endothelial cell proliferation or migration and allows for better reendothelialization after injury compared to PCBs (5).

Liquid delivery devices (LDDs) are a novel drug delivery approach that eliminate the risk of balloon coating wash-off through local administration of a liquid therapeutic. LDDs isolate a treatment zone using proximal and distal occlusion balloons and has a built-in pressure sensor to monitor the pressure-dependent delivery of the drug into the tissue. Preclinical studies with this device have shown that Apt14 can be delivered directly to the medial layer of arteries and retained for up to 24 hours (6).

Thus far, no studies have explored liquid delivery of Apt14 under AVF hemodynamics. We have developed a clinically relevant ex vivo bioreactor system that simulates the AVF hemodynamic environment and enables evaluation of endovascular devices such as LDDs. The overarching goal of this study is to explore feasibility of Apt14 as a treatment for VNH in AVFs using this ex vivo AVF bioreactor. Specifically, we will optimize Apt14 delivery to maximize retention in arterial and venous tissue under AVF conditions. **We hypothesize that liquid delivery of Apt14 will be an effective and safer alternative to PCBs for treating VNH in AVFs.** We will test this hypothesis with the following Aim:

Aim #1: To elucidate conditions that maximize VSMC-targeted Apt14 delivery with the liquid delivery device using an ex vivo AVF bioreactor system. The delivery pressure and drug concentration will be varied to determine the optimal delivery conditions for maximal Apt14 retention in arterial and venous tissue under AVF hemodynamics. Acute tissue retention of Apt14 will be analyzed at 1- and 24-hours using quantitative reverse-transcription polymerase chain reaction (qRT-PCR). The optimal delivery conditions will also be evaluated qualitatively through visualization of a fluorescent formulation of Apt14 (Alexa594-Apt14) via fluorescent microscopy.

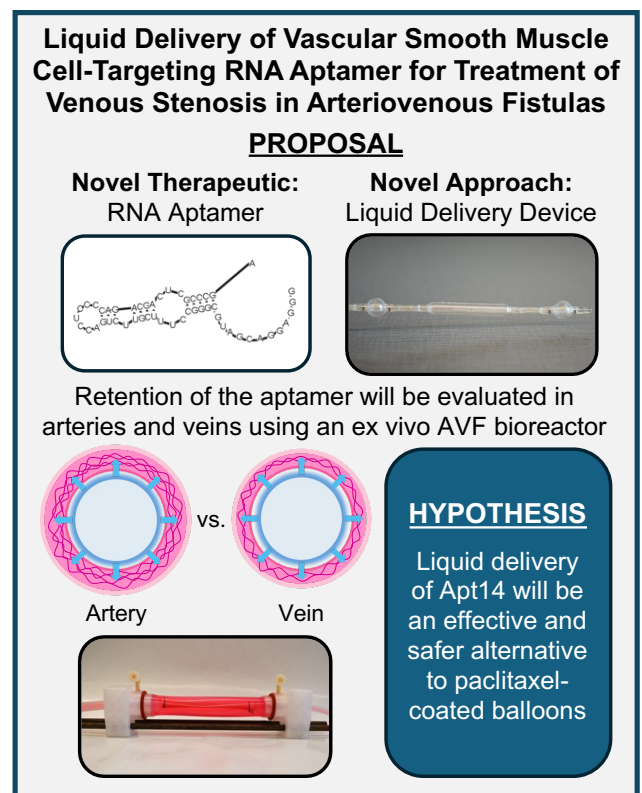


Figure 1. Graphical abstract of proposed study.

SIGNIFICANCE

End-stage renal disease (ESRD) is an advanced stage of chronic kidney disease for which the only treatments are hemodialysis or kidney transplant. For patients receiving hemodialysis, maintaining functional vascular access is critical. The most commonly used vascular access is the arteriovenous fistula (AVF), used by almost 65% of hemodialysis patients in the U.S. While AVFs have the lowest infection and failure rates compared to other vascular access options (1), they are not without their complications. On average, approximately 30% of AVFs require intervention to maintain patency 6 months after starting hemodialysis (1) and 40-60% experience restenosis one year after the primary intervention.

The main cause of loss of patency is venous neointimal hyperplasia (VNH), which is characterized by increased vessel wall stiffness and decreased luminal diameter. VNH is caused by an accumulation of vascular smooth muscle cells (VSMCs) and extracellular matrix (ECM) in the intima, resulting from increased expression of fibrotic signals by endothelial cells in response to vascular injury (7). One source of this vascular injury is the creation of the AVF which exposes the vein to higher blood flow, wall shear stress, and pressure when the artery is joined.

The current recommended treatment for VNH in AVFs, according to the Kidney Disease Outcomes Quality Initiative (KDOQI), is mechanical dilation using high-pressure balloon angioplasty (8). However, as balloon angioplasty can cause additional injury to the vessel endothelium, this often results in restenosis of the treated lesion. In coronary and peripheral arteries, angioplasty-associated restenosis is treated with paclitaxel-coated balloons (PCBs). These devices are low-pressure angioplasty balloons that are coated with the anti-proliferative drug paclitaxel. This has shown overwhelming success in coronary and peripheral applications, thus PCBs are being explored as a treatment option for VNH in AVFs. Several clinical trials have investigated the use of PCBs for VNH, but many studies – including two major multi-center randomized controlled trials (9,10) – have produced conflicting results of the treatment efficacy of PCBs.

PCBs deposit paclitaxel-drug carrier particles on the vessel endothelium when the balloon is inflated. This treatment method has a couple of limitations, the first being reliance on drug coating adhesion to the lumen and the diffusion of the drug into the tissue. This allows delivery efficacy to be affected by blood flow wall shear stress, which can cause drug loss due to particle wash-off. Another limitation of this method is the lack of specificity of paclitaxel. This drug can also inhibit proliferation of endothelial cells and thereby prevent reendothelialization after PCB intervention. These issues underscore the need for development of better treatments of AVF stenosis.

INNOVATION

Use of a liquid drug delivery device to treat AVF stenosis

A novel delivery approach utilizing liquid delivery devices (LDD) offers several advantages over PCBs, including localized delivery of drugs directly into the tissue rather than deposition on the luminal surface. The LDD that will be used in this study is the occlusion perfusion catheter (Advanced Catheter Therapies, Chattanooga, TN) (**Figure 2**), as it has demonstrated deeper tissue drug delivery than other commercially available LDDs (11). This LDD has two occlusion balloons that create an isolated treatment zone and has a built-in fiber optic pressure transducer that allows the delivery pressure to be monitored. In this study, drug delivery with this LDD will be evaluated for the first time in venous tissue and under AVF hemodynamics.

Use of a novel RNA aptamer to treat AVF stenosis

RNA aptamers have binding affinities and specificities comparable to antigen-antibody interactions. Aptamer 14 (Apt14) inhibits VSMC migration by blocking platelet-derived growth factor receptor- β (PDGFR- β) phosphorylation and disrupting the phosphoinositide 3-kinases (PI3K)/Akt pathway (3). It has been investigated in murine and porcine in vivo models and has demonstrated decreased neointimal development and improved reendothelialization compared to PCBs (4,5). In peripheral arterial ex vivo models, Apt14 has demonstrated retention in arterial tissue for up to 24 hours (6). This study proposes the first investigation of Apt14 in ex vivo venous tissue under AVF hemodynamics.

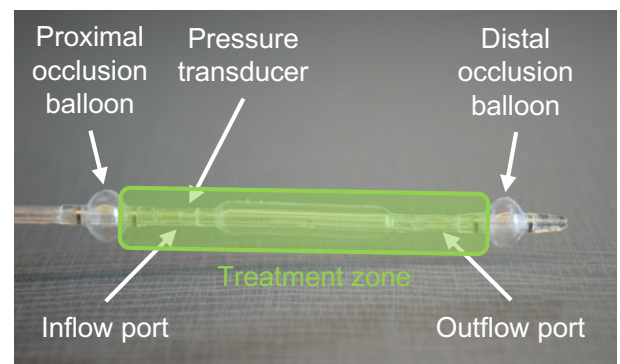


Figure 2. Diagram of liquid delivery device. Treatment zone is created by inflation of the occlusion balloons. Pressure transducer reports intra-luminal delivery pressure.

RESEARCH STRATEGY

AIM #1: TO ELUCIDATE CONDITIONS THAT MAXIMIZE VSMC-TARGETED APTAMER DELIVERY WITH THE LOCAL LIQUID DELIVERY DEVICE USING AN EX VIVO BIOREACTOR SYSTEM.

Rationale and Overview

Previous studies have evaluated use of LDDs in peripheral arterial benchtop models and have demonstrated successful retention of liquid therapies, such as Apt14 (12,13). These studies showed that delivery efficacy and acute drug retention are primarily dependent by drug concentration and delivery pressure. In Task 1a, we will test various Apt14 concentrations and delivery pressures to determine the optimal conditions that maximize retention of Apt14 at 1- and 24-hours, the time points for evaluating acute delivery efficacy. In Task 1b, we will qualitatively evaluate the optimal delivery conditions using a fluorescent formulation of Apt14 (Alexa594-Apt14) to visualize retention and depth of drug penetration into the tissue.

Task 1A: Optimization of Liquid Delivery Conditions That Maximize Tissue Retention of Apt14

Experimental Design

AVF Bioreactor

These studies will be performed in an ex vivo AVF bioreactor (**Figure 3**). Porcine carotid arteries and jugular veins harvested from a local abattoir will be used as the test vessels. The circulation medium will be Dulbecco's Modified Eagle's Medium containing low glucose, L-glutamine, 110 mg/L sodium pyruvate, pyridoxine hydrochloride 10% fetal bovine serum, and 1% antibiotic-antimycotic. The vessel housing will be a transparent silicone-polymer tube containing agarose gel which enables measurement of the vessel diameter and flow rate via ultrasound (**Figure 4**). A signal generator will create an input waveform that is used by a gear pump to create the pulsatile flow through the bioreactor system. The average flow rate and systolic/diastolic pressures measured in this bioreactor are 665 ± 85 mL/min and 146/87 mmHg (**Figure 5**). In contrast, the average arterial flow in a peripheral arterial bioreactor ranges from 15 to 150 mL/min with systolic/diastolic pressures of 120/80 mmHg (14).

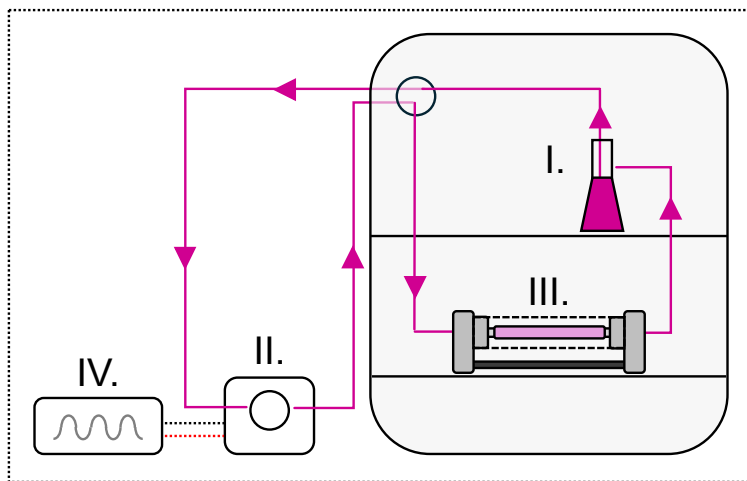


Figure 3. Schematic of ex vivo AVF bioreactor system. Pink lines and arrows indicate direction of fluid flow.

I. Circulation medium reservoir III. Vessel housing
II. Gear pump IV. Signal generator

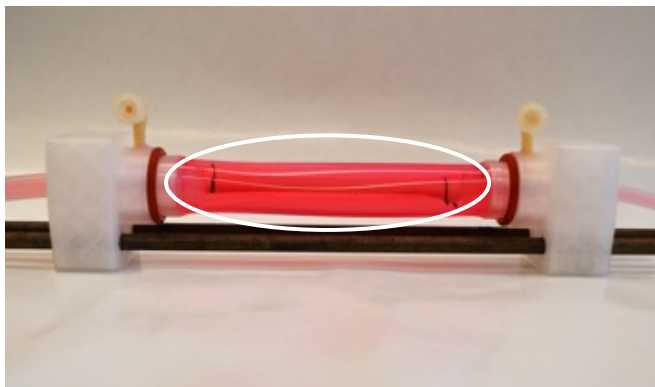


Figure 4. Vessel housing in AVF bioreactor with arterial sample (circled).

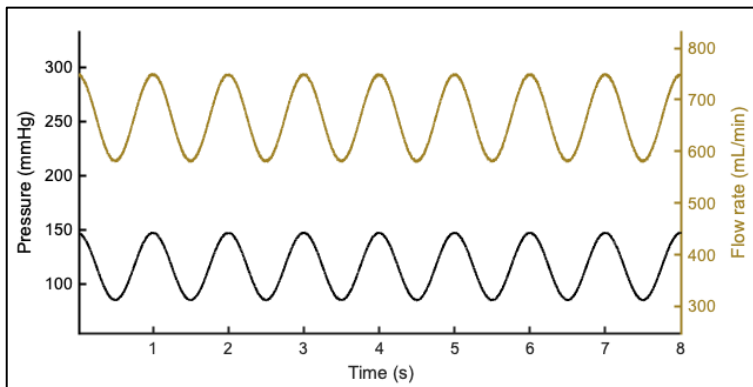


Figure 5. Average flow rate (665 ± 85 mL/min) and systolic/diastolic pressure (146/87 mmHg) in bioreactor.

Apt14 Delivery

The Apt14 solution will be prepared at 100, 250, and 500 nM, as described previously (6) (**Table 1**). These concentrations were selected based on an in vitro arterial VSMC migration assay in which cellular migration was found to be dose-dependent (**Figure 6**) (4).

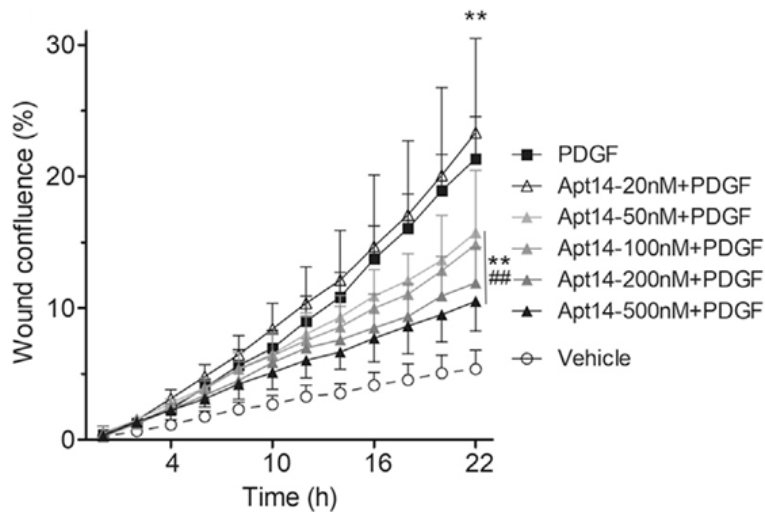


Figure 6. Dose-dependency of arterial VSMC migration on Apt14 concentration (4).

Before aptamer delivery, the ex vivo vessels will undergo vascular injury with an angioplasty balloon catheter inflated to a 1:1.15 artery-to-balloon overstretch ratio. To deliver the aptamer to the ex vivo vessels, the LDD will be inserted into the bioreactor system over a guidewire and the occlusion balloons deployed to obstruct flow of the circulation media. The aptamer solution will be used to flush media from treatment zone, after which the outflow port of the device will be closed and the aptamer delivered for 2 minutes, as this is the average clinical inflation time of PCBs (**Figure 7**). The chosen delivery pressures, 0.1 and 0.4 atm, were determined based on a mathematical and experimental model of liquid delivery in arterial tissue as the minimal and maximal pressures needed to deliver liquid therapeutic agents with this LDD (12,15).

Table 1. Breakdown of experimental conditions and required number of samples for Task 1a.					
Vessel Type	Delivery Pressure	Apt14 Conc.	# per Group	Time Points	# of Samples
Artery	0.1 and 0.4 atm	100, 250, 500 nM	4	1, 24 hours	48
Vein	0.1 and 0.4 atm	100, 250, 500 nM	4	1, 24 hours	48
					Total: 96

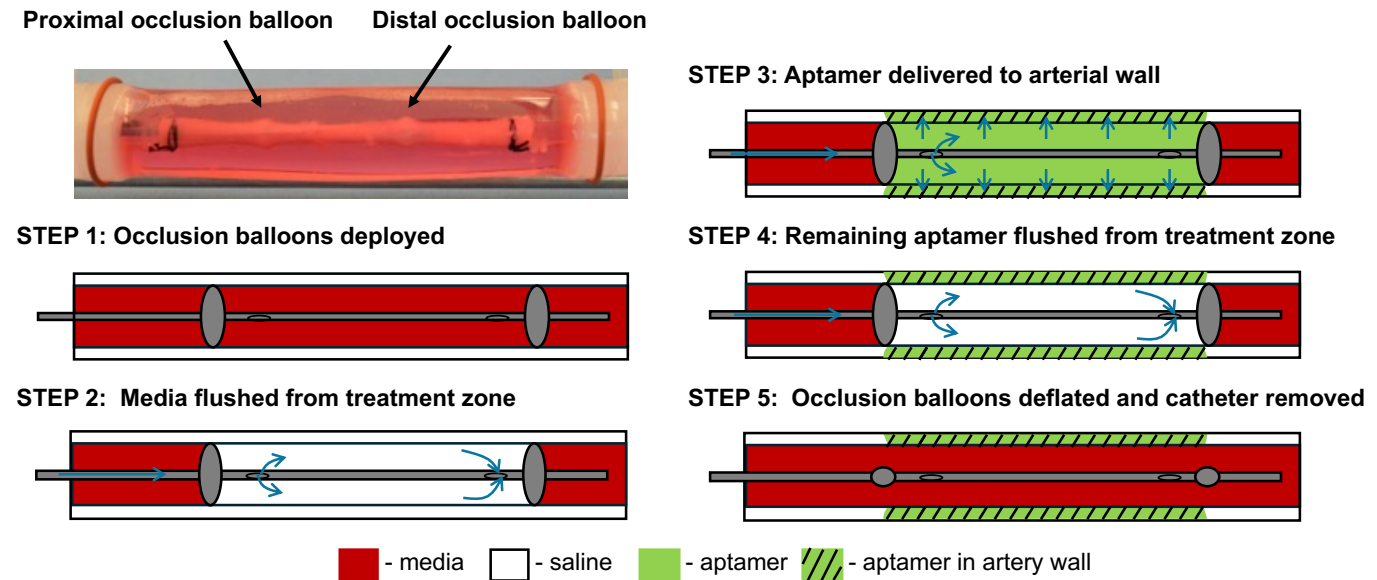


Figure 7. Schematic illustration of liquid delivery device.

Quantification of Apt14 tissue retention

The samples will be evaluated at 1- and 24-hours for acute drug retention using quantitative reverse-transcription polymerase chain reaction (qRT-PCR) as previously described (6). The treated samples will be removed from the bioreactor system and placed in a lysis buffer for overnight incubation. A known quantity of Sel1 processing control aptamer (M12-23) will be added to the lysate. The aptamer will be recovered by TRIzol extraction, measured by qRT-PCR, and normalized to the recorded tissue mass.

Expected Results

Previous studies of Apt14 in peripheral arterial bioreactors confirm that liquid delivery of Apt14 can result in drug retention for up to 24-hours. The proposed task will help identify the delivery conditions that maximize retention of Apt14 at 1- and 24-hours in arterial and venous tissue under AVF hemodynamics. We expect the AVF bioreactor to mimic the physiological conditions of an AVF in vivo and Apt14 retention in the vessel to increase with greater drug concentrations and delivery pressure. We also expect the concentration of retained drug to be greater in arteries than veins given the thicker medial, VSMC layer in arteries.

Alternative Approaches

We do not anticipate complications quantifying Apt14 from the tissue samples using qRT-PCR. However, should problems arise, an alternative quantification technique – aptamer fluorescence binding and internalization (AFBI) assay – can be used with the fluorescent formulation of Apt14, Alexa594-Apt14 (16).

Task 1b: Validation of optimal liquid delivery conditions via fluorescent microscopy visualization

Experimental Design

Alexa594-Apt14 Delivery

A fluorescent formulation of Apt14 (Alexa594-Apt14) will be used to prepare a aptamer solution as described previously (6) at two of the optimal concentrations determined above in Task 1a. Similar to the delivery procedure described in Task 1a, the ex vivo vessels will be injured prior to aptamer delivery using an angioplasty balloon catheter. The LDD will be used to deliver the Alexa594-Apt14 solution for 2 minutes at the optimal delivery pressure determined in Task 1a (**Table 2**).

Table 2. Breakdown of experimental conditions and required number of samples for Task 1b.					
Vessel Type	Delivery Pressure	Apt14 Conc.	# per Group	Time Points	# of Samples
Artery	Optimal pressure from Task 1a	Top two optimal conc. from Task 1a	4	1, 24 hours	16
Vein	Optimal pressure from Task 1a	Top two optimal conc. from Task 1a	4	1, 24 hours	16
					Total: 32

Visualization of Apt14 tissue retention and penetration

The retention and depth of penetration of Alexa594-Apt14 will be visualized using fluorescent microscopy as previously described (11) at 1- and 24-hours (**Figure 8**). The treated samples will be removed from the bioreactor system and sectioned into 5-mm segments and frozen in optical cutting temperature (OCT) compound. 10- μ m cross-sections will be cut using a cryostat and sections will be mounted to microscope slides and imaged using a fluorescent microscope. Depth of drug penetration will be measured from the internal elastic lamina to the maximum penetration depth and normalized to the thickness of the medial layer or wall thickness.

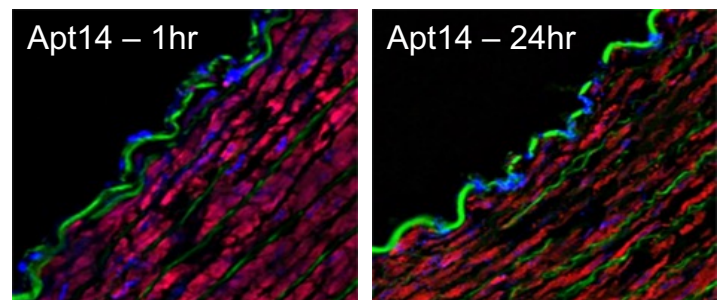


Figure 8. Fluorescent microscopy imaging of aptamers (in blue), smooth muscle cells (in red), and elastic fiber (in green) (6).

Expected Results

The proposed task will help visualize the optimal delivery conditions that maximize retention of Apt14 in arterial and venous tissue under AVF hemodynamics. We expect the quantitative results derived in Task 1a to be supported by this qualitative evaluation via fluorescent microscopy of Alexa594-Apt14.

Alternative Approaches

We do not anticipate any complications visualizing Alexa594-Apt14 in the tissue. Completion of this study will support further investigation of the use of LDDs and Apt14 for treating venous stenosis in arteriovenous fistulas, including evaluation of this novel therapy in porcine models.

REFERENCES

1. DePietro DM, Trerotola SO. Choosing the right treatment for the right lesion, part I: a narrative review of the role of plain balloon angioplasty in dialysis access maintenance. *Cardiovasc Diagn Ther* [Internet]. 2023 Feb 28 [cited 2023 May 23];13(1):21232–21232. Available from: <https://cdt.amegroups.com/article/view/104822>
2. DePietro DM, Trerotola SO. Choosing the right treatment for the right lesion, Part II: a narrative review of drug-coated balloon angioplasty and its evolving role in dialysis access maintenance. *Cardiovasc Diagn Ther* [Internet]. 2023 Feb 28 [cited 2023 May 23];13(1):23359–23259. Available from: <https://cdt.amegroups.com/article/view/107866>
3. Thiel WH, Esposito CL, Dickey DD, Dassie JP, Long ME, Adam J, et al. Smooth Muscle Cell-targeted RNA Aptamer Inhibits Neointimal Formation. *Mol Ther* [Internet]. 2016 Apr [cited 2021 Sep 6];24(4):779–87. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4886937/>
4. Yazdani SK, Lei B, Cawthon CV, Cooper K, Huett C, Giangrande PH, et al. Local intraluminal delivery of a smooth muscle-targeted RNA ligand inhibits neointima growth in a porcine model of peripheral vascular disease. *Mol Ther - Nucleic Acids* [Internet]. 2022 Sep 13 [cited 2022 Oct 24];29:577–83. Available from: <https://www.sciencedirect.com/science/article/pii/S2162253122002037>
5. Lei B, Liu LB, Stokes L, Giangrande PH, Miller FJ, Yazdani SK. Smooth muscle cell-targeted RNA ligand promotes accelerated reendothelialization in a swine peripheral injury model. *Mol Ther - Nucleic Acids* [Internet]. 2023 Dec 12 [cited 2023 Sep 21];34. Available from: [https://www.cell.com/molecular-therapy-family/nucleic-acids/abstract/S2162-2531\(23\)00236-6](https://www.cell.com/molecular-therapy-family/nucleic-acids/abstract/S2162-2531(23)00236-6)
6. Udofot O, Lin LH, Thiel WH, Erwin M, Turner E, Miller FJ, et al. Delivery of Cell-Specific Aptamers to the Arterial Wall with an Occlusion Perfusion Catheter. *Mol Ther - Nucleic Acids* [Internet]. 2019 Jun 7 [cited 2021 Sep 6];16:360–6. Available from: [https://www.cell.com/molecular-therapy-family/nucleic-acids/abstract/S2162-2531\(19\)30063-0](https://www.cell.com/molecular-therapy-family/nucleic-acids/abstract/S2162-2531(19)30063-0)
7. Shiu YT, Rotmans JI, Geelhoed WJ, Pike DB, Lee T. Arteriovenous conduits for hemodialysis: how to better modulate the pathophysiological vascular response to optimize vascular access durability. *Am J Physiol-Ren Physiol* [Internet]. 2019 May 1 [cited 2021 Sep 10];316(5):F794–806. Available from: <https://www.physiology.org/doi/10.1152/ajprenal.00440.2018>
8. Lok CE, Huber TS, Lee T, Shenoy S, Yevzlin AS, Abreo K, et al. KDOQI Clinical Practice Guideline for Vascular Access: 2019 Update. *Am J Kidney Dis* [Internet]. 2020 Apr 1 [cited 2023 Mar 30];75(4, Supplement 2):S1–164. Available from: <https://www.sciencedirect.com/science/article/pii/S0272638619311370>
9. Trerotola SO, Lawson J, Roy-Chaudhury P, Saad TF. Drug Coated Balloon Angioplasty in Failing AV Fistulas: A Randomized Controlled Trial. *Clin J Am Soc Nephrol* [Internet]. 2018 Aug 7 [cited 2021 Sep 6];13(8):1215–24. Available from: <https://cjasn.asnjournals.org/content/13/8/1215>
10. Lookstein RA, Haruguchi H, Ouriel K, Weinberg I, Lei L, Cihlar S, et al. Drug-Coated Balloons for Dysfunctional Dialysis Arteriovenous Fistulas. *N Engl J Med* [Internet]. 2020 Aug 20 [cited 2021 Sep 6];383(8):733–42. Available from: <https://doi.org/10.1056/NEJMoa1914617>
11. Todd M, Liu LB, Saul JM, Yazdani SK. Pre-clinical investigation of liquid sirolimus for local drug delivery. *Front Cardiovasc Med* [Internet]. 2023 Sep 15 [cited 2023 Sep 15];10. Available from: <https://www.frontiersin.org/articles/10.3389/fcvm.2023.1184816>
12. Atigh MK, Turner E, Christians U, Yazdani SK. The use of an occlusion perfusion catheter to deliver paclitaxel to the arterial wall. *Cardiovasc Ther* [Internet]. 2017 Aug [cited 2021 Sep 6];35(4):10.1111/1755-5922.12269. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5511576/>
13. Cawthon CV, Cooper K, Huett C, Lloret A, Villar-Matamoros E, Stokes L, et al. Pre-Clinical Investigation of Liquid Paclitaxel for Local Drug Delivery: A Pilot Study. *Pharmaceuticals* [Internet]. 2020 Nov 28 [cited 2021 Sep 6];13(12):434. Available from: <https://www.mdpi.com/1424-8247/13/12/434>
14. Cooper K, Cawthon CV, Goel E, Atigh M, Christians U, Yazdani SK. The Development of an ex vivo Flow System to Assess Acute Arterial Drug Retention of Cardiovascular Intravascular Devices. *Front Med Technol* [Internet]. 2021 Jun 10 [cited 2021 Sep 6];3:675188. Available from: <https://www.frontiersin.org/articles/10.3389/fmedt.2021.675188/full>
15. Atigh MK, Goel E, Erwin M, Greer R, Ohayon J, Pettigrew RI, et al. Precision delivery of liquid therapy into the arterial wall for the treatment of peripheral arterial disease. *Sci Rep* [Internet]. 2021 Sep 21 [cited 2022 Apr 11];11(1):18676. Available from: <https://www.nature.com/articles/s41598-021-98063-z>
16. Thiel WH, Giangrande PH. AFBI assay – Aptamer Fluorescence Binding and Internalization assay for cultured adherent cells. *Methods* [Internet]. 2016 Jul [cited 2022 Aug 2];103:180–7. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S1046202316300391>

RESOURCES AND FACILITIES

Personnel: The Yazdani laboratory currently has one graduate student research assistant and two lab technicians.

Laboratory: The Yazdani laboratory is located on the 4th floor of Wake Downtown, the new, state-of-the art facility dedicated to interdisciplinary research and teaching at Wake Forest University. Dr. Yazdani's space is designed to perform research programs focused on cardiovascular mechanics. There is approximately 1,100 square feet of wet-laboratory space well equipped to conduct the proposed experiments. Bench space, sinks, fume hood, vacuum and pressurized air, and CO₂ are present.

Equipment: The Yazdani laboratory space includes a cell culture hood (LABGard Class II, Type A2), one constant Volume Fume Hood (Hamilton SafeAire II), four CO₂ cell culture incubator (Heratherm), refrigerator (Isotemp), -20 and -80 degrees freezer (Fisher Scientific), an inverted light microscope with camera (Echo Rebel Hybrid), an inverted fluorescent microscope with camera (Echo Revolve), two cryostats (Leica and Thermo Scientific), and a centrifuge (Ohaus Frontier 5000). The thermal cycler (SimpliAmp Thermal Cycler) and PCR system (GeneAmp PCR System 9700) that will be used for PCR are located in the biology and biochemistry laboratory spaces on the 2nd floor of Wake Downtown.