# Ex Vivo Perfusion Research in a Transplant Setting

Subjects: Transplantation

Contributor: Carolina Campos Pamplona , Cyril Moers , Henri G. D. Leuvenink , L. Leonie van Leeuwen

Given the importance of renal vasculature and the consequences of vascular injury during renal transplantation, it would be extremely helpful if the condition of the renal vasculature could be assessed and potentially improved before transplantation. Ex vivo perfusion, whether it is hypothermic (HMP) or normothermic (NMP), may offer the possibility to assess kidney function prior to transplantation and could provide a treatment platform in a controlled and isolated environment. Therefore, investigating and assessing vascular damage during machine perfusion could provide a better understanding of its underlying mechanisms, which could potentially be translated into assessment or treatment techniques.

kidney transplantation ex vivo perfusion endothelial cells vascular viability

## 1. Real-Time Assessment of Vasoactivity during NMP

A healthy endothelium is responsible for modulating the vascular tone according to pressure, flow, and oxygenation conditions. Signals from endothelial cells (ECs) are received by the smooth muscle cells (SMCs) to either relax or contract, therefore creating vasodilation and vasoconstriction. An impaired vasomotor response can be the result of either dysfunction in endothelial signaling to smooth muscle cells or primary dysfunction of SMCs. Therefore, it is important to assess both endothelial-dependent and -independent vasoactive responses [1][2][3]. It would be valuable to explore different techniques known from other fields of research. For example, there are several methods for examining endothelial viability and capillary dysfunction in cardiovascular research [2] that could be implemented in combination with ex vivo perfusion with just some minor adjustments. One of the most common techniques used to assess endothelial-dependent function involves monitoring the vasomotor response to a certain stimulus, while the lack of or diminished response would be an indicator of poor function [2].

### 1.1. Endothelial Vasoactive Response Evaluation

A simple technique that could be implemented during NMP is to administer boluses of vasoactive substances during perfusion and observe flow variations. Those drugs could be directed at a certain vessel layer or vasoactive pathway of interest, and viable vessels should be capable of responding to such stimuli. A more accurate assessment could be achieved by, for example, using ultrasound to measure vessel diameter or laser speckle contrast imaging (LSCI) to observe changes in perfusion profiles [2][4]. In 2018, Bath et al. [5] reported that long warm ischemia times (WIT) directly affect vasodilation capacity after administering vasoactive boluses during NMP, suggesting irreversible endothelial injury, and cold ischemia time (CIT) further diminished that potential. The study

indicates that influencing vasoactivity during perfusion could provide a measurement of vascular injury and, therefore, viability.

## 1.2. Iontophoresis

Another technique that could be implemented during NMP is iontophoresis, which assesses the nitric oxide (NO) availability in the microvasculature [2][6]. While similar to the previously mentioned technique, iontophoresis is a less systemic approach and has not yet been adapted for an ex vivo setting. In the clinic, the principle consists of placing two watertight chambers on the forearm skin that conduct an electrical current to transfer positive or negatively charged vasoactive agents through resistance vessels locally, and the delivery rate depends on the density and strength of the current. Acetylcholine and sodium nitroprusside are the most common agents used in this technique. To measure the vascular changes, laser doppler flowmetry (LDF) or laser doppler imaging (LDI) can be used. LDF and LDI are similar techniques, differing on the size of the scanned area—LDF measures perfusion over a single point, and LDI scans a whole area [2]. With some adaptation and validation, this technique could be implemented during NMP to assess the dynamic vascular response to vasoactive stimuli. In this way, evaluation can be performed in a localized manner without affecting the whole organ, and it would offer real-time results. In addition to doppler techniques, LSCI could be an alternative option for imaging, as it has high special and temporal resolution and has already been validated in perfusion models for microvascular perfusion assessment [4].

## 1.3. Venous Occlusion Plethysmography

Venous occlusion plethysmography could also be applied during NMP with appropriate adaptation/validation. Normally, this technique is used in the forearm, and it entails stopping venous return while there is arterial inflow. This flow occlusion causes the forearm to increase in volume over time in proportion to the incoming arterial flow [2][7]. Two strain gauges are placed high on the forearm and the wrist, one to cause venous occlusion and one to exclude the hand from the measurement. An automated device (plethysmograph) is connected to the forearm cuff and controls the in- and deflation of the gauges respecting the maximum venous filling. The increase in forearm volume is picked up by the machine, as the increase in length of the strain gauge causes a change in electrical resistance, therefore representing an increase in forearm blood flow [2]. In principle, healthy resistance vessels should be able to retain more flow by inducing vasodilation and quickly respond to the strain gauge removal and restore venous return by adjusting vasodilation [7]. This technique could be seen as more aggressive compared to others, but if validated, it could still provide extra real-time information on vascular adaptation capacity during perfusion when testing different protocols or drugs, as it might correlate with other parameters.

## 1.4. Arterial Stiffness

Arterial stiffness is also a principal factor to consider when studying vascular viability [2][8][9]. In vivo, with each heartbeat, pressure waves travel through the vasculature, and compliant arteries dampen these pressure oscillations to deliver blood flow in a smooth manner. At branching points, these pressure waves are reflected, and the stiffer an artery is, the faster these waves travel back. Increased stiffness can be caused by reduced NO production by the endothelial cells, loss of SMC tone, excessive collagen deposition in the vessel wall, and

atherosclerosis [2][8]. There are a few techniques available to assess arterial stiffness, the most common being pulse wave analysis (PWA) and pulse wave velocity (PWV), which have been previously associated with coronary microvascular endothelial function [2][10]. Both methods are similar, only differing in the number of measured locations (PWA uses a single point, and PWV uses two points simultaneously). The arterial pressure wave is measured with a transducer by flattening an artery—but not occluding it. Then, the difference between peak readings is calculated as a percentage, and the higher the number, the stiffer the artery. In an NMP setup, a transducer could be easily placed either on the renal artery or on the silicone tubing from the circuit to acquire PWA or PWV data, and arterial stiffness could potentially be measured during perfusion [2]. However, measurement readings would still need to be calibrated and validated for the setup.

# 2. Exploring New Techniques to Unravel Molecular Mechanisms of Vascular Injury

Most of the assessment techniques explored above focus on the real-time contractility of the renal vasculature. However, there is still a knowledge gap in general ex vivo physiology, and there are currently no predictive biomarkers for ex vivo renal viability [11]. Since vasculature stability is crucial for maintaining homeostasis and optimal renal function, new techniques should be explored to further understand and investigate its (patho)physiology in an ex vivo manner, as in vivo and ex vivo vascular responses might differ considerably. Although cumbersome, more elaborate and extensive techniques should not be overlooked, as they might be key to unveiling basic ex vivo vascular physiology so that, in the future, they can be simplified and perhaps correlated to more real-time parameters currently in use.

## 2.1. Quantifying Endothelial Cell Shedding with Flow Cytometry

Previous studies have shown that EC damage derived from renal graft injury causes EC shedding, leading to an increase in circulating endothelial cells (cEC) in blood, which correlates with plasma markers such as vWF and E-selectin [12]. During machine perfusion, the presence of cECs could potentially be an indicator of vascular injury. The major advantage of measuring cECs is that their presence is not dependent on EC activation (measured by vWF and E-selectin levels). In that sense, endothelial damage can be differentiated from dysfunction and could therefore aid in graft viability monitoring [12].

Various human and murine studies have used flow cytometry to identify endothelial cells, and a plethora of markers have been studied to identify cells coming from different tissues and vessel sizes [12][13][14][15]. The main antibody that is used to detect cECs in human blood is CD146, but other markers such as CD31, CD144, CD146, CD105, Lectins, VEGF-2, PV-1, vWF, Tie-2/TEK, and HLA-II could be used to phenotype endothelial cells further down to their location within the kidney and vessel size [12][13][14][15]. However, it is important to note that different (donor) kidneys can express these markers in different proportions [14], thus making analysis a complicated process to understand and standardize.

So far, no studies have been performed to investigate the presence of cEC as a vascular viability assessment during machine perfusion. One of the reasons could be that many experimental transplantation studies are performed in pigs due to their similar physiology to humans. Porcine endothelial cells have barely been studied, and the general availability of porcine antibodies is scarce [12]. For flow cytometry analysis, (renal) endothelial-specific pre-labeled primary porcine antibodies are inexistent. Secondly, NMP experiments followed by transplantation are limited due to high costs and little availability of centers for porcine research. As for the clinical situation, quantifying cECs in HMP perfusate is easily applicable as HMP is already standard clinical care. However, since NMP is only barely implemented clinically, flow cytometry during NMP will take some time to initiate. Furthermore, most perfusion studies do not generate post-transplant data as the kidneys are not transplanted, hence correlating cEC numbers with transplant outcomes has not been established.

## 2.2. Lightsheet Fluorescence Microscopy

Lightsheet fluorescence microscopy (LSFM) is an ex vivo tissue microscopy technique, and its imaging principle consists of horizontal fluorescent tissue excitation and vertical scanning [16]. This technique enables optical sectioning of the sample while still in a three-dimensional architecture, therefore enabling whole organ imaging in small animal models. Murine, zebrafish, and organoid models are the most commonly used LSFM during analysis, and their organs of interest vary (hearts, lungs, kidneys, eyes, etc.) [17][18][19][20][21][22][23][24][25]. Several studies have been performed to assess renal vascular viability and architecture using murine models [21][25][26].

During HMP or NMP, the administration of antibodies against endothelial markers—such as CD31, lectins, and glycocalyx components—could potentially help identify the vasculature during LSFM and highlight areas of shedding [14][15][21][25][26]. Additionally, this technique could be used to identify inflammatory activation/infiltration sites along the vessels and overall vascular architecture and even offer insight into capillary rarefaction [26]. As seen in **Table 1**, the need for tissue fixation limits this technique to retrospective analysis, but it could still be of great help in studying the localized vascular impact of machine perfusion and its potential treatment targets. Since whole porcine and human organs do not fit in the imaging chamber of the microscope, biopsies could be collected and analyzed using LSFM.

**Table 1.** Summarized view of previous applications and (dis)advantages of suggested techniques.

| Technique | Previous Application | Advantages | Disadvantages |
|---|---|---|---|
| Response to vasoactive stimuli | Cardiovascular research and ex vivo perfusion | Real-time evaluation | Affects the whole organ |
| Iontophoresis | Clinically used | Real-time evaluation; local effect | Requires validation and placement of chambers on the kidney surface |
| Ultrasound/Laser doppler | Clinically used | Already clinically used; Real-time evaluation; | No generalized assessment possible; Microvasculature not |

| Technique | Previous Application | Advantages | Disadvantages |
|---|---|---|---|
| | | | visible; Requires placement of probe on kidney surface; |
| Laser speckle imaging | Ex vivo perfusion | Real-time evaluation; Already validated during ex vivo perfusion; microvasculature more visible | Needs a specific dark chamber for scanning; Only superficial visualization is possible |
| Venous occlusion plethysmography | Clinically used | Real-time evaluation; No need to administer drugs | More aggressive; Affects the whole organ by occlusion of outflow |
| Arterial stiffness | Clinically used | Real-time evaluation; No need to administer drugs; Could possibly be measured in the tubing | Requires validation and possible placement of probes on the kidney surface |
| Flow cytometry | Human, murine studies | Specific analysis/labeling of desired cells/proteins | Not a real-time assessment; Antibody availability could be difficult; |
| Lightsheet microscopy | Murine, zebrafish, organoid studies, etc. | Three-dimensional analysis of sample; Specific analysis/labeling of desired cells/proteins | Not a real-time assessment; Not possible to do whole organ analysis in large animal/human models; Sample needs to be fixed and cleared; Lengthy |
| Microcomputed tomography | Murine in vivo and ex vivo | Three-dimensional analysis of sample; Possible to visualize small structures; Not lengthy; In vivo scanning in small animal models | Not a real-time assessment; Not possible to perform functional analysis |
| Magnetic resonance imaging | Clinically used and ex vivo perfusion | Possible to perform anatomical and functional analysis; Already applied to ex vivo perfusion; Real-time assessment | Still under study for interpretation during ex vivo perfusion; Logistically cumbersome |
| Targeted treatment and nanoparticles | Human, porcine, and murine studies, ex vivo perfusion | Treatment is delivered to the isolated organ; Nanoparticles allow long-term drug delivery | No long-term assessment is possible during ex vivo perfusion; Drug dosage needs validation and tested for toxicity |
| siRNA and/or miRNA treatment | Cardiovascular research, murine and human studies, and ex vivo perfusion | Treatment is delivered to the isolated organ; Treatment can have an effect even after transplantation | No long-term assessment possible during ex vivo perfusion; Drug dosage needs validation and tested for toxicity |

| Technique | Previous Application | Advantages | Disadvantages |
|---|---|---|---|
| Precision-cut slices | Murine, porcine, and human studies, ex vivo perfusion studies | Long-term assessment possible; Multiple drugs can be tested in a single organ | Full organ functionality not possible; Prone to infections |
| Fibrinolysis | Human and porcine transplant studies, ex vivo perfusion | Treatment is delivered to the isolated organ; Avoids further graft damage | Post-transplant consequences need more extensive study |

## 2.3. Imaging Renal Microvascular Architecture with Microcomputed Tomography

Microcomputed tomography (micro-CT) is a relatively new technology that can be applied to image samples inside and out in a non-destructive way and acquire high-resolution images with fast 3D cross-section reconstruction. This technique has been applied in in vivo studies for the assessment of longitudinal treatment effects [27] and in ex vivo research studies analyzing various organs, such as renal microvasculature architecture imaging and glomerular quantification [25][28]. Voxel sizes can be adjusted to image microvasculature in rodent and porcine models, and structures as small as afferent/efferent arterioles can be visualized. Studies have reported that early structural changes can be detected by micro-CT and vascular rarefaction, increased microvascular density, and microvascular remodeling have been correlated to early stages of pathological processes such as polycystic kidney disease, hypercholesterolemia, and increased oxidative stress in stenotic kidneys, respectively [25]. In addition, alterations in vascular molecular mechanisms, such as expression of HIF-1α ad VEGF have been associated with changes in vascular structure in polycystic kidneys after micro-CT imaging [25][28].

The advantages of micro-CT are that full geometric structures can be analyzed, glomerular and peritubular capillaries can be distinguished, and the spatial density and tortuosity of vascular beds can be visualized in a relatively fast manner [25][27][28]. However, unlike other imaging modalities, no functional assessments can be performed; this technique requires radiation, and without any modifications to the machine, kidneys cannot be imaged while perfused. Therefore, this technique would be restricted as a post-perfusion analysis in porcine and human discarded kidney models, but observed changes could be correlated to other molecular and/or real-time parameters. In rodent transplantation studies involving ex vivo perfusion as a platform for targeted treatment, micro-CT has the potential to evaluate in vivo vascular responses longitudinally for post-transplant monitoring, therefore offering insight into outcomes.

## 2.4. Visualizing Renal Perfusion Profile with Magnetic Resonance Imaging

Magnetic resonance imaging is a technique commonly used in the clinic for non-invasive real-time organ morphological and functional assessment. Several studies published by Schutter et al. [29][30] reveal that functional MRI (fMRI) imaging during renal NMP can be used to unveil renal ex vivo physiology and as a reliable and independent viability assessment tool. The combination of NMP and fMRI could prove of extreme value to


4. Heeman, W.; Maassen, H.; Calon, J.; Van Goor, H.; Leuvenink, H.G.D.; van Dam, G.M.; Boerma, E.C. Real-time visualization of renal microperfusion using laser speckle contrast imaging. J. Biomed. Opt. 2021, 26, 056004.
5. Bath, M.F.; Hosgood, S.A.; Nicholson, M.L. Vasoreactivity to Acetylcholine During Porcine Kidney Perfusion for the Assessment of Ischemic Injury. J. Surg. Res. 2019, 238, 96–101.
6. Nagar, R.; Senger, S. A simple user-made iontophoresis device for palmoplantar hyperhidrosis. J. Cutan. Aesthet. Surg. 2016, 9, 32.
7. Wilkinson, I.B.; Webb, D.J. Venous occlusion plethysmography in cardiovascular research: Methodology and clinical applications. Br. J. Clin. Pharmacol. 2001, 52, 631–646.
8. Scandale, G.; Dimitrov, G.; Recchia, M.; Carzaniga, G.; Minola, M.; Perilli, E.; Carotta, M.; Catalano, M. Arterial stiffness and subendocardial viability ratio in patients with peripheral arterial disease. J. Clin. Hypertens. 2018, 20, 478–484.
9. Saito, M.; Okayama, H.; Nishimura, K.; Ogimoto, A.; Ohtsuka, T.; Inoue, K.; Hiasa, G.; Sumimoto, T.; Higaki, J. Possible link between large artery stiffness and coronary flow velocity reserve. Heart 2008, 94, e20.
10. Wilkinson, I.B.; Hall, I.R.; MacCallum, H.; Mackenzie, I.S.; McEniery, C.; van der Arend, B.J.; Shu, Y.; MacKay, L.S.; Webb, D.J.; Cockcroft, J.R. Pulse-wave analysis: Clinical evaluation of a noninvasive, widely applicable method for assessing endothelial function. Arterioscler. Thromb. Vasc. Biol. 2002, 22, 147–152.
11. Hamelink, T.L.; Oguntu, B.; de Beule, J.; Lantinga, V.A.; Pool, M.B.F.; Veenma, I.H.; Leuvenink, H.G.D.; Joles, J.A.M.; Moers, C. Renal Normothermic Machine Perfusion: The Road Toward Clinical Implementation of a Promising Pretransplant Organ Assessment Tool. Transplantation 2022, 106, 269–279.
12. Post, J.C.J.H.; Weenink, R.P.; Van Wijk, A.C.W.A.; Heger, M.; Böing, A.N.; van Hulst, R.A.; van Gulik, T.M. Characterization and quantification of porcine circulating endothelial cells. Xenotransplantation 2013, 20, 18–26.
13. Lammers, R.G.M.; Lagendijk, L.M.; Tiller, G.; Dam, W.A.; Lancaster, H.L.; Dana, M.R.; Seelen, M.A.; Hepkema, B.G.; Pol, R.A.; Leuvenink, H.G.D. Machine-perfused donor kidneys as a source

investigate interactions in the transplant process (vision 2021, 5, 21; 6 of 7) as well as opening doors to adding an extra set of variables to organ quality prediction models.

During NMP, fMRI acquires information on vascular parameters such as general vessel architecture and regional blood flow. Arterial labeling (ASL) techniques, as mentioned, track the magnetic properties of blood through the kidneys, as is shown in Murine C. et al., 2021 [30]. Unexpectedly, as an unexpected result from Schutter's experiments, ex vivo perfused kidneys showed low cortical flow and high medullary flow for the first two hours, after which it returned to in vivo physiological characteristics. These data highlight once more how different kidneys might behave in vivo and ex vivo. With that in mind, further research into vascular-specific fMRI sequences is needed to investigate and understand renal ex vivo responses, and results should be carefully interpreted.

## 3. Treating Vascular Injury in an Ex Vivo Setting

Last but not least, ex vivo machine perfusion can provide a unique opportunity to treat kidney grafts whilst outside of the body. Machine perfusion itself may already offer superior protection against IRI-related vascular damage, but specific drug targeting to repair or prevent additional injury could further improve the preservation of (suboptimal) donor kidneys.

### 3.1. Targeting Hypoxia via the HIF Pathways

An inevitable consequence of ischemia and organ storage is tissue hypoxia, which is most commonly activated via the HIF pathways. With vascular dysfunction and damage, vasoconstriction, inflammation, and thrombosis are phenomena that further lead to low oxygen delivery to the tissue [31][32]. The HIF signaling cascade is intended to protect the organ from excessive hypoxic damage, but it has been reported that continuous and non-specific activation of HIF pathways can have detrimental consequences. As hypoxia response is very heterogenous even within the same organ—and since the kidney is the most vulnerable to hypoxia damage—it is essential to investigate further the importance of these renal responses in an ex vivo machine perfusion setting.

Several attempts have been made to counteract hypoxia by pre-treating the donor in an experimental setting. Animal studies have reported that pre-treatment of donors by enhancing HIF-1α or inhibiting PHD has a positive effect on post-transplant outcomes, and human observational studies indicated that non-rejected and better-functioning grafts presented with higher HIF-1α levels post-transplant [32][33][34]. However, in a clinical setting, pre-treatment of the donor is very difficult for ethical reasons. Therefore, an ex vivo model could be of significant impact on the ectopic studying and conditioning of kidneys prior to transplantation by directly or indirectly enhancing HIF-1α activity. In 2017, a study by Hollis et al. [35] used renal HMP to deliver PHD inhibitors to investigate whether there was an anaerobic metabolic shift after ischemic injury. Even though a normothermic functional assessment of the kidneys was not performed in the study, the group could show that inhibitor administration during HMP alone could alter the metabolic phenotype of the kidneys. To our knowledge, PHD inhibitors have not yet been tested in a normothermic machine perfusion setup. This would be relevant to examine as PHD inhibitor kinetics could work best under physiological temperatures and, therefore, more protective for the kidney vasculature.

of human renal endothelial cells. Am. J. Physiol. Ren. Physiol. 2021, 320, F947–F962.
14. Grant, D.; Wanner, N.; Frimel, M.; Erzurum, S.; Asosingh, K. Comprehensive phenotyping of endothelial cells using flow cytometry 2: Human. Cytom. Part A 2021, 99, 257–264.
15. Grant, D.; Wanner, N.; Frimel, M.; Erzurum, S.; Asosingh, K. Comprehensive phenotyping of endothelial cells using flow cytometry 1: Murine. Cytom. Part A 2021, 99, 251–256.
16. Adams, M.W.; Loftus, A.F.; Dunn, S.E.; Joens, M.S.; Fitzpatrick, J.A.J. Light Sheet Fluorescence Microscopy (LSFM). Curr. Protoc. Cytom. 2015, 71, 394.
17. Puelles, V.G.; Combes, A.N.; Bertram, J.F. Clearly imaging and quantifying the kidney in 3D. Kidney Int. 2021, 100, 780–786.
18. Zhang, B.; Gao, Y.; Zhang, S.; Yu, W.; Zhang, S.; Wang, Z.; Chen, L.; et al. Light-sheet fluorescence imaging charts the gastrula origin of vascular endothelial cells in early zebrafish embryos. Cell. Discov. 2020, 6, 74.
19. Ding, Y.; Ma, J.; Langenbacher, A.D.; Baek, K.I.; Lee, J.; Chang, C.; Hsu, J.J.; Kulkarni, R.P.; Belperio, J.; Shi, W.; et al. Multiscale light-sheet for rapid imaging of cardiopulmonary system. JCI Insight 2018, 3, e121396.
20. Pranist, C.; Ashrafzadeh, P.; Mead, T.; Figueiredo, A.; Chang, K.; Richardson, D.; Venkaraman, L.; Richads, M.; Russo, A.M.; Harrington, K.; et al. Mouse retinal cell behaviour in space and time using light sheet fluorescence microscopy. eLife 2020, 9, e49779.
21. Farber, G.; Parks, M.M.; Lustgarden Gurahmich, N.L.; Zhang, Y.; Monette, S.; Blanchard, S.C.; Di Lorenzo, A.; Blobel, C.P. ADAM10 controls the differentiation of the coronary arterial endothelium. Angiogenesis 2019, 22, 237–250.
22. Held, M.; Santeramo, I.; Wilm, B.; Murray, P.; Levy, R. Ex vivo live cell tracking in kidney organoids using light sheet fluorescence microscopy. PLoS ONE 2018, 13, e0199918.
23. Kluza, E. Shedding 3D light on endothelial barrier function in arteries. Atherosclerosis 2020, 310, 91–92.
24. Merz, S.F.; Korste, S.; Bornemann, L.; Michel, L.; Stock, P.; Squire, A.; Soun, C.; Engel, D.R.; Detzer, J.; Lorchner, H.; et al. Contemporaneous 3D characterization of acute and chronic myocardial I/R injury and response. Nat. Commun. 2019, 10, 2312.
25. Apelt, K.; Bijkerk, R.; Lebrin, F.; Rabelink, T.J. Imaging the renal microcirculation in cell therapy. Cells 2021, 10, 1087.
26. Klingberg, A.; Hasenberg, A.; Ludwig-Portugall, I.; Medyukhina, A.; Männ, L.; Brenzel, A.; Engel, D.R.; Figge, M.T.; Kurts, C.; Gunzer, M. Fully automated evaluation of total glomerular number and capillary tuft size in nephritic kidneys using lightsheet microscopy. J. Am. Soc. Nephrol. 2017, 28, 452–459.

### 3.2. Targeting Angiogenesis via the VEGF Pathway

Another potential vascular treatment pathway is the VEGF pathway. In the face of tissue hypoxia and cellular injury, the vasculature turns to angiogenesis in an attempt to repair its structure and restore homeostasis, and this process is tightly regulated between proangiogenic and antiangiogenic factors [35]. The VEGF pathway has been a focus of various studies in recent years, and enhancement of this pathway in early ischemic processes has shown promise in maintaining vascular viability [36][37]. Once ex vivo angiogenic physiology is unraveled, treatment of kidneys prior to transplantation during perfusion could be an interesting approach to avoid chronic injury. With this method, similar to HIF pathway interventions, donor treatment is avoided and the organ would be treated individually without systemic effects. Target agents could be administered more easily or even associated with microparticles for a longer local effect post-transplant.

### 3.3. Targeting Coagulation with Fibrinolytics

In a healthy setting, assessment of renal flow distribution patterns during ex vivo perfusion is an interesting approach to detect early damage to ECs, thereby impairing their protective mechanisms against thrombosis [38]. Indeed, this dysfunction, making the vascular environment prone to those processes. The exposition of the EC basal membrane activates vWF and factor XII, which stimulates the extrinsic and intrinsic coagulation pathways, respectively. From then on, glycoprotein receptors promote platelet aggregation, and platelet degranulation catalyzes it even further [1][38]. Putting effort into exploring inflammatory and coagulation pathways ex vivo could improve current perfusion protocols to avoid extreme endothelial activation, which can be greatly beneficial for maintaining homeostasis and avoiding further graft damage. As an example, a few previous porcine and human transplant studies have shown that inducing fibrinolysis ex vivo during flush or HMP to actively remove clots from the circulation and prevent further endothelial damage was beneficial for organ functioning, thus improving graft viability post-transplant [39][40].

### 3.4. Using siRNA and miRNA to Attenuate Inflammation

Since endothelial cells are responsible for initiating inflammatory, immunologic, coagulatory, and even graft rejection processes [1][38][41][42], modifying ECs during ex vivo perfusion by reducing or even inhibiting the expression of certain pathways can benefit organ (vascular) viability without further compromising the recipient's system [43]. This (temporary) cellular reprogramming could possibly be achieved by the administration of small interfering RNA (siRNA), microRNA (miRNA), or even pharmacological agents [44][45].

A study by Cui et al. [44], for example, pre-treated human epicardial coronary arteries with siRNA-loaded biodegradable poly(amine-co-ester) (PACE) nanoparticle against class II transactivator (CIITA) via NMP. CIITA is a positive regulator for the transcription of class II major histocompatibility complex (MHC-II) molecules, and it induces temporary unresponsiveness to interferon (IFN)-γ-mediated induction of MHC-II molecules. Isolated arteries were perfused for 6 hours, and CIITA knockdown was established. After 6 hours of transplant, pre-treated arteries showed a significantly lower expression of MHC-II molecules and IFN-γ-induced vascular cell adhesion molecule-1 (VCAM-1) compared to non-treated arteries. Afterward, these vessels were transplanted into immunodeficient mice, with a focus on the von Willebrand Factor and Factor VIII and V. After 6 weeks of transplant, pre-


26. Kelly, K.A.; Allport, J.R.; Tsuzuki, A.; Weissleder, R. Fluorescence Molecular Tomography of Cathepsin Activity Imaging in Myocardial Infarction. Ann. Biomed. Eng. 2005, 33, 1188–1194.
27. Kavvadas, P.; Anastasiou, I.; Dussaule, J.C.; Chatziantoniou, C. Angiotensin II Activates Notch1 Signaling in Proximal Tubular Cells to Promote Inflammation and Fibrosis. Nephrol. Dial. Transplant. 2009, 24, 2387–2396.
28. Bentley, M.D.; Rodriguez-Porcel, M.; Lerman, A.; Sarafov, M.H.; Romero, J.C.; Pelaez, L.I.; Grande, J.P.; Ritman, E.L.; Lerman, L.O. Enhanced renal cortical vascularization in experimental hypercholesterolemia. Kidney Int. 2002, 61, 1056–1063.
29. Schutter, R.; van Varsseveld, O.C.; Lantinga, V.A.; Pool, M.B.F.; Hamelink, T.L.; Potze, J.H.; Leuvenink, H.G.D.; Laustsen, C.; Borra, R.J.H.; Moers, C. Magnetic resonance imaging during warm ex vivo kidney perfusion. Artif. Organs. 2023, 47, 105–116.
30. Schutter, R.; Lantinga, V.A.; Hamelink, T.L.; Pool, M.B.F.; van Varsseveld, O.C.; Potze, J.H.; Hillebrands, J.; van den Heuvel, M.C.; Dierckx, R.A.J.O.; Leuvenink, H.G.D.; et al. Magnetic resonance imaging assessment of renal flow distribution patterns during ex vivo hypothermic machine perfusion in porcine and human kidneys. Transpl. Int. 2021, 34, 1643–1655.
31. Marsboom, G.; Rehman, J. Hypoxia signaling in vascular homeostasis. Physiology 2018, 33, 328–337.
32. Shu, S.; Wang, Y.; Zheng, M.; Liu, Z.; Cai, J.; Tang, C.; Dong, Z. Hypoxia and Hypoxia-Inducible Factors in Kidney Injury and Repair. Cells 2019, 8, 207.
33. Hollis, A.; Patel, K.; Smith, T.; Nath, J.; Tennant, D. Manipulating the HIF Pathway in Renal Transplantation, Current Progress and Future Developments. J. Clin. Exp. Transplant. 2016, 1, 110.
34. Debuisch, R.; Thuillier, R.; Le Pape, S.; Rossard, L.; Jayle, C.; Billault, C.; Goujon, J.M.; Hauet, T. Effects of warm ischaemia combined with cold preservation on the hypoxia-inducible factor 1α pathway in an experimental renal autotransplantation model. Br. J. Surg. 2014, 101, 1739–1750.
35. Tanaka, T.; Nangaku, M. Angiogenesis and hypoxia in the kidney. Nat. Rev. Nephrol. 2013, 9, 211–222.
36. Leonard, E.C.; Friedrich, J.L.; Basile, D.P. VEGF-121 preserves renal microvessel structure and ameliorates secondary renal disease following acute kidney injury. Am. J. Physiol. Ren. Physiol. 2008, 295, F1648–F1657.
37. Hara, A.; Wada, T.; Furuichi, K.; Sakai, N.; Kawachi, H.; Shimizu, F.; Shibuya, M.; Matsushima, K.; Yokoyama, H.; Egashira, K.; et al. Blockade of VEGF accelerates proteinuria, via decrease in nephrin expression in rat crescentic glomerulonephritis. Kidney Int. 2006, 69, 1986–1995.
38. Pablo Moreno, J.A.D.; Serrano, J.C.; Revuelta, I.; Sánchez, M.J.; Lirias, A.T.; Vascular Endothelium and Coagulation Homeostasis, Disease and Treatment, with a Focus on the Von Willebrand Factor and Factors VII and V. Int. J. Mol. Sci. 2022, 23, 8283.

### 3.5. Targeting Fibrosis via the TGF-β Pathway

The last step of renal injury is fibrosis. This process is the result of irreparable damage to the vasculature and renal tissue, in which TGF-β plays a key role. Attenuating fibrosis during renal ex vivo perfusion could be an interesting approach to prevent its onset due to persistent inflammation after IRI and to allow the vasculature to repair and re-vascularize prior to the no-return point. Avoiding vascular injury and rarefaction is crucial to avoiding further fibrinogenesis.

Drugs to target such pathways could be easily administered in an HMP or NMP perfusate [46]. However, fibrosis is a slow process that cannot be observed within only a few hours, so other techniques need to be explored in addition to machine perfusion alone. Recent studies by van Furth et al. [47] and van Leeuwen et al. [46] have proposed the use of precision cut kidney slices as a model for the long-term evaluation of drug effect and IRI molecular mechanisms. This model has the benefit of being able to use a single organ to test various drugs simultaneously, and it is also an upgrade from cell cultures as it maintains part of the tissue's architecture, therefore allowing evaluation of cell-cell and cell-matrix interactions. Using this technique after administering drugs during ex vivo perfusion could open doors to investigating long-term effects (24–48 h) of fibrosis in renal tissue. Ultimately, they have shown that fibrosis can be attenuated by targeting the TGF-β pathway.


38. Zulpaite, R.; Miknevicius, P.; Leber, B.; Strupas, K.; Stiegler, P.; Schemmer, P. Ex vivo Kidney Machine Perfusion: Therapeutic Potential. Front. Med. 2021, 8, 808719.

39. Brasile, L.; Stubenitsky, B.M.; Booster, M.H.; Arenada, D.J.; Kootstra, G. Overcoming severe renal ischemia: the role of ex-vivo warm perfusion. Transplant. Proc. 2005, 37, 3505–3508.

40. Olausson, M.; Antony, D.; Travnikova, G.; Johansson, M.; Nayakawde, N.B.; Banerjee, D.; Søfteland, J.M.; Premaratne, G.U. Novel Ex-Vivo Thrombolytic Reconditioning of Kidneys Retrieved 4 to 5 Hours after Circulatory Death. Transplantation 2022, 106, 1577–1588.

41. Stanek, A.; Fazeli, B.; Bartuś, S.; Sutkowska, E. The Role of Endothelium in Physiological and Pathological States: New Data. Biomed. Res. Int. 2018, 2018, 1098039.

42. Rubanyi, G.M. The role of endothelium in cardiovascular homeostasis and diseases. J. Cardiovasc. Pharmacol. 1993, 22, S1–14.

43. Cui, J.; Qin, L.; Zhang, J.; Abrahimi, P.; Li, H.; Li, G.; Tietjen, G.T.; Tellides, G.; Pober, J.S.; Saltzman, W.M. Ex vivo pretreatment of human vessels with siRNA nanoparticles provides protein silencing in endothelial cells. Nat. Commun. 2017, 8, 191.

44. Simonson, B.; Das, S. MicroRNA Therapeutics: The Next Magic Bullet? Mini Rev. Med. Chem. 2015, 15, 467–474.

45. Hanna, J.; Hossain, G.S.; Kocerha, J. The potential for microRNA therapeutics and clinical research. Front. Genet. 2019, 10, 478.

46. van Leeuwen, L.L.; Leuvenink, H.G.D.; Olinga, P.; Ruigrok, M.J.R. Shifting Paradigms for Suppressing Fibrosis in Kidney Transplants: Supplementing Perfusion Solutions with Anti-fibrotic Drugs. Front. Med. 2022, 8, 2917.

47. van Furth, L.A.; Leuvenink, H.G.D.; Seras, L.; de Graaf, I.A.M.; Olinga, P.; van Leeuwen, L.L. Exploring Porcine Precision-Cut Kidney Slices as a Model for Transplant-Related Ischemia-Reperfusion Injury. Transplantology 2022, 3, 139–151.


Retrieved from https://encyclopedia.pub/entry/history/show/105324