

Ewout's Oxygen Kidney Protocol

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1 Introduction

1.1 Background

A dramatic shortage of donor organs has led to an increased use of extended-criteria and donation after circulatory death donor grafts. Respectively, these are donor grafts older than 60 or in some situations 50 years old and donor grafts that have experienced a loss of blood flow. These types of donor organs are more susceptible to ischemia-reperfusion injury (IRI) [1, 2]. IRI is damage caused by reperfusion after a period of restriction of blood flow called ischemia or lack of oxygen. This has led to a growing interest in research aimed at oxygenation during machine perfusion (MP). Oxygenation is a tool used in MP research to decrease the risk of IRI. This project aims at providing researchers with a new tool to pursue their research in oxygenation during MP.

Currently oxygenation typically happens at supraphysiological levels, which are levels higher than normally found in the body. Only few studies were designed to compare different oxygenation settings within models, even though non-excessive oxygen delivery might be of utmost benefit during normothermic machine perfusion (NMP) [3, 4]. This discrepancy might be the consequence of a lack of available tools that allow controlled oxygen levels.

Also, it appears to be the case that there is no default oxygen supply requirement by grafts. Instead it should be tailored for specific needs of grafts of different quality, this includes kidneys [3]. This knowledge emphasizes the need of a system that can deliver different amounts of oxygen.

Additionally, one can hypothesize that specific needs of kidneys change during MP. Perhaps induced by chemical substances supplemented by the researcher, a change in the environment such as temperature or any other change in the metabolic rate. A tool that can dynamically change to the current requirements of the kidney could potentially be a solution to enhance the health of the kidney during MP.

1.2 Goal

Using the development surrounding the previous experiments a prototype was built. This prototype has the potential to monitor and control the oxygen level in the perfusate. However, it missing key information regarding the transfer function of the controller and its corresponding influence. Therefore, the goal of this experiment is to find the corresponding constants in regards to the PID-control.

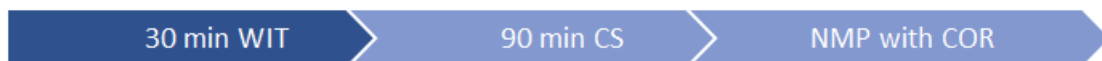


Figure 1: Course of MP actions, warm ischemic time (WIT), cold storage (CS), normothermic machine perfusion (NMP), controlled oxygenated rewarming (COR).

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3 Materials Slaughterhouse

3.1 Blood

- 5L beaker
- 5L barrel
- 5ml/25000 IE Heparine (Apotheek; 14179857)
- 5ml syringe
- 20G needle

3.2 Kidneys

- 2x Gauze (10x10cm)
- Surgical Blade
- 2x Surgical scissors (sharp!)
- 2x Surgical forceps
- 2x Surgical clamps
- 1L NaCl for flush (bottle versylene NaCl 0.9% Fresenius >sterile; Inkoop; 15226352)
- Syringe 60 ml (wond/blaaspuit)
- Normal 60 ml syringe
- Catheter (5cm) for flush
- 2x 10ml syringe
- 2x 5ml syringe
- 2x 20G needle
- Ice box (for inspecting the kidneys)
- Gloves
- Trash bags
- Pen + Case Report Form (CRF)

3.3 Cold storage

- Styrofoam box
- Ice (to fill bottom of box)
- UW-CS

4 Method slaughterhouse

4.1 Before leaving the lab

1. Fill Styrofoam box with ice
2. Collect UW-CS and NaCl from the cold room
3. Pack slaughterhouse bags (see materials)

4.2 Blood

1. Put the Heparin (25000 IE) in the 5L beaker with the syringe.
2. The butcher takes the beaker to the animal and takes approximately 2-3 liters of blood from the pig.
3. Pour the blood in a 5L barrel.

4.3 Kidneys

1. Put a trash bag around the ice box to protect it from the blood.
2. Fill the ice box with ice and place a gauze on top. Use a syringe and some NaCl to wet the gauze.
3. The butcher brings the kidney pair. Lay them down on the table with the aorta facing upwards.
4. Throughout the entire preparation, make sure to keep the veins as intact as possible. The vein will have to be cannulated in the lab.
5. Cut the aorta lengthwise in half and search the renal arteries. Check the renal arteries for branching. Choose the kidney with no branching or branching closest to the kidney. Locate the ureter.
6. Remove the contralateral kidney.
7. Remove excessive fat.
8. When the kidney is all prepared for the next steps, take a photo.
9. Fill the 60 ml syringe with cold NaCl and attach the catheter. Check for arterial leakage.
10. After 30 minutes of WIT. Place kidneys on ice and start flush. Place the catheter carefully in the renal artery and flush slowly. Be careful not to apply excessive pressure with the syringe!
11. Use a total of 180 ml for the flush.
12. Remove the catheter.

4.4 Cold storage

1. Put some UW-CS solution in the smallest organ bag. Put the kidney in the bag and make sure that the kidney is surrounded by the solution.
2. Add a second bag for possible leakage.
3. Put the whole package in the styrofoam box and make sure that ice is surrounding the total package for optimal cooling.

5 Materials lab

5.1 Perfusate

Perfusion solution	Amount
Ringers Lactate	280 mL
Amoxicillin/Clavulaanzuur (1000 mg/200 mg)	1 ampul
8.4% Sodium Bicarbonate	10 mL
5% glucose	10 mL
Dexamethasone (20 mg/ml)	333 ul
Leukocyte depleted blood	500 mL
Sodium Nitroprusside (20 mg/ml) ADD LAST MINUTE!	100 uL
Insulin (100 IU/ml)	0,186 ml

Table 1: Perfusate composition.

- Catheter bag (2L)
- Funnel
- Leukocyte filter (BioR O2 plus BS PF, Fresenius Kabi, Bad Homburg, Germany; A2BB0080)
- Tie wraps
- Silicone tubing to connect catheter bag to leukofilter
- Clamps
- Parafilm

5.2 Normothermic machine perfusion

- Ewout's oxygen prototype + flow sensor
- SophistiKate + pump + pressure sensor + organ chamber + temperature sensor
- Heater and thermostat
- Corresponding tubing for the above three items
- Laptop
- Centrifugal pump head (Deltastream DP3, MEDOS Medizintechnik AG, Stolberg, Germany)
- Clamp-on flow sensor (ME7PXL clamp, Transonic Systems Inc., Ithaca, NY)
- Oxygenator with integrated heat exchanger (HILITE 1000, MEDOS Medizintechnik AG, Stolberg, Germany)
- Heat exchanger water bath (Julabo MP-5, Labortechnik, Seelbach, Germany)
- Tie wraps
- 500 ml leukocyte depleted blood
- Sample line (for blood gas)
- 5ml tubes (perfusate samples)
- 2 PreSens Fibox 4
- Carbogen and nitrogen gas fitting including corresponding pressure reducing valves

6 Method lab

6.1 Preparing perfusate

1. Attach the leukocyte filter to the outlet of the catheter bag. And attach a funnel to the top of the tubing.
2. Put the tie wraps to the top sides of the catheter bag.
3. Close all the tubing underneath the bag and fill the bag with approximately 1L of blood.
4. Fill the leukocyte filter and de-air by holding it upside down.
5. Attach the system to a suspension hook and place the beaker underneath. Attach the tube to the beaker with the red clip.
6. Open the outlet of the catheter bag and de-air the leukocyte filter, by keeping it upside down until blood leaves the filter.
7. When the blood has passed the filter, close the clamp.
8. Combine the leukocyte depleted blood with the remaining perfusate solutions components, excluding the sodium nitroprusside. See table 1.

6.2 Perfusion circuit

1. The entire setup should be placed in the fume chamber.
2. Put the heater and thermostat in the fume chamber. Make sure to put heater on oscillating.
3. Connect the following components using corresponding tubing in this order: oxygenator blood outlet > PreSens oxygen sensor > pressure sensor > blood sample line > arterial cannula > kidney > venous cannula > PreSens oxygen sensor > let leak in water bath > pump head > flow sensor > blood inlet oxygenator.
4. Connect the sensors to the corresponding SophistiKate or Ewout's Oxygen prototype, consecutively connect those to the laptop.
5. Load corresponding software.
6. Connect heat exchanger to oxygenator.
7. Place the SophistiKate temperature sensor in the organ chamber, in the perfusate.
8. Connect the carbogen and nitrogen to the prototype.
9. Adjust the pressure of the carbogen such that a maximum flow of ... l/min is achieved. Use the built in flow sensor (not the rotameter) to read the flow.
10. Using the rotameter, adjust the flow of the nitrogen to a static 0.5 l/min.
11. Using the prototype, achieve a 133 hPa arterial oxygen pressure.

6.3 Normothermic machine perfusion

1. Add the perfusate to the perfusion circuit. Make sure no air remains in the circuit.
2. Prime the pressure sensor and the blood sample line.
3. Take the kidney out of the CS.
4. Place a cannula inside the ureter and tie 2-0 braided suture around the distal end of ureter to make sure it remains in the same place.
5. Tie suture around the ureter cannula to complete fixation.
6. Place a cannula inside the renal artery, secure it with suture.
7. *In similar fashion, cannulate the vein.*
8. Flush kidney with 50 ml NaCl for the removal of UW-CS and check for leakage.

9. Add the sodium nitroprusside to the perfusion solution in the circuit.
10. Place the prepared kidney in the organ chamber.
11. Check if the system is still free of air bubbles. If not, remove them.
12. Using the SophistiKate, find a pressure that achieves around 150-200 ml/min of perfusate flow. Do not exceed 120 mmHg.
13. Do not use sinusoidal pressure.
14. Fill up the arterial cannula with perfusion solution and connect kidney to the perfusion circuit. Make sure to keep the system and kidney air free.
15. Pause the pump.
16. Calibrate pressure sensor by clicking on calibrate pressure sensor. This takes a couple of seconds.
17. Resume the pump.
18. Fill up the arterial cannula with perfusion solution and connect to the perfusion circuit. Make sure to keep the system air free.
19. Click on timer and start data log immediately after. Fill in the histology number as the file name. Do NOT open the excel files during the experiment!
20. Close the fume chamber.

6.4 Controlled oxygenated rewarming

1. Perfuse at room temperature for a total of 15 minutes.
2. Put the thermostat and water bath heat exchanger to 29°C.
3. Once the temperature is achieved, perfuse for 15 minutes.
4. Put the thermostat and water bath heat exchanger to 37°C.
5. Wait until temperature is achieved.

7 References

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