An ex-vivo perfused porcine liver for magnetic resonance imaging

A master thesis

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Symbols and abbreviations

- HCC = hepatocellular carcinoma
- MRI = magnetic resonance imaging
- CT = computed tomography
- SPECT/CT = single-photon emission computed tomography
- HIFU = high-intensity focused ultrasound (HIFU)
- US = ultrasound imaging
- PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-Analyses
- NMP= normothermic machine perfusion
- HA = hepatic artery
- PV = portal vein
- RL = Ringer's lactate
- KH = Krebs-Henseleit buffer
- EC = Euro-Collins solution
- UW = University of Wisconsin solution
- WIT = warm ischemia time
- CIT = cold ischemia time
- ID = inner diameter
- O_2 = oxygen
- PO_2 = partial oxygen pressure
- CO_2 = carbon dioxide
- PCO_2= partial carbon dioxide pressure
- HCO_3 = bicarbonate
- BE = base excess
- sO_2 = oxygen saturation
- Na = sodium
- K = potassium
- iCa = ionised calcium
- Glu = glucose
- Hct = hematocrit
- PCV = partial cell volume
- Hb = hemoglobin
- $T1$ = T1 relaxation time, time constant for regrowth of longitudinal magnetisation
- $T2$ = T2 relaxation time, time constant for decay of transverse magnetisation
- T_2^* = observed T2* relaxation time, including magnetic field inhomogeneities
- $STPF$ = scaled tissue perfusion factor
- R_2^* = T2* relaxation rate
- $[Gd]$ = concentration of gadolinium-based contrast agent
- S = signal intensity
- TE = echo time
- k = proportionality factor

Preface

With my BME master track focusing on imaging, I was looking for a practical master thesis project that would combine medical imaging with system design. Ever since performing my bachelor thesis at M3i, I have had a great interest in this research group, so I was excited to hear that they had so many master projects to offer. When I learned about the Liver-Twin project I was instantly enthusiastic and found this project a great match for me.

I have certainly learned a lot during this master thesis. I was very eager to find out all of the parameters involved in isolated organ perfusion, so within the first few weeks, we decided to turn my basic literature search into a systematic literature review. It was very interesting to delve into so many different aspects, learning about the technical aspects of the existing perfusion setups and also learning about the elements that affect liver function.

After several months of theoretical research, I was excited to start doing practical work. By comparing different pieces of equipment and contacting the manufacturers, I learned a lot about the pumps and sensors that we used in the setup. It was also very interesting to work with animal organs and blood, even though things got messy sometimes. Finally implementing the system in the MRI was the highlight of the experimental work and it was very rewarding to see the setup in action and to create some amazing MRI results at the same time.

I would like to express my gratitude to all of the people that have supported me throughout this project. Starting off, I would like to thank the people of M3i and the students in TL1332A with which I shared many coffee breaks and had many fun and interesting conversations.

I would like to thank Judith Brands for helping me state the query for the literature review and for her tips for evaluating the papers.

I would also like to thank Ana Martins Costa and Lisa Herzog for their help during the many Monday-morning trips to the slaughterhouse and for their help regarding the equipment for working with blood.

My thanks also go out to some of the technicians, Johan van Hespen and Wim Leppink, for helping me with questions about sensors and equipment and for letting us use the lab for the liver perfusion experiments.

I want to thank Jaap Greve and Remco Liefers for helping obtain great MRI results. It was fascinating to visualise blood flow through the liver with these MRI scans.

My special thanks go out to Tess Snoeijink. Your expertise in liver preparations was very valuable for this project. You gave very useful tips on how to work with organs and perfusion setups during our many meetings and you were a great help during the liver perfusion experiments.

I would like to express my gratitude to my supervisors. Firstly, I would like to thank Srirang Manohar. Discussing my results with you always inspired and motivated me and you always reminded me to keep the big picture in mind.

Secondly, thank you Jutta Arens for your advice regarding the system design and working with blood. We had many useful discussions during our meetings together and those have helped me a lot in designing and optimising the system.

My gratitude also goes out to my daily supervisor Erik Groot-Jebbink. I appreciate how I could always come to you for advice and you always made time to answer my questions. You also provided very useful feedback on this thesis, helping me to write concisely and to the point. I learned a lot from your practical way of thinking.

A very special thank you goes out to Jan van der Hoek for the pleasant collaboration on this project. We took this journey together, starting off with the systematic literature review and we built and tested the system together. It was great to see what we achieved in the months that we worked on the system.

I wish the Liver-Twin team the best of luck on improving this system and I am looking forward to seeing what this field of research will bring in the future.

Finally, this preface would not be complete without thanking my parents and my friends for their moral support and for proofreading my thesis.

Abstract

The distribution of microspheres throughout the liver during radioembolisation can be studied using modalities such as magnetic resonance imaging (MRI), aiming to improve the efficacy of this treatment for treating hepatocellular carcinoma. The preferred platform for such studies is an isolated perfused animal liver since it provides access to the dynamic behaviour of the liver while being able to control input blood flow. Clinical systems exist which are able to maintain the function of donor organs for several hours until transplantation. However, these lack the control of certain perfusion parameters and they cannot always be tuned to medical imaging modalities. The aim of this research was therefore to design, develop, and implement an in-house system that is able to keep porcine livers viable while performing magnetic resonance imaging scans.

A systematic literature review was performed to gain insights into the existing isolated liver perfusion systems that are used in combination with medical imaging. This review resulted in an overview of descriptions of the perfusion setups, physiological conditions required for maintaining liver function — describing parameters such as flow rate, pressure, perfusate type, and temperature — methods of testing liver viability, and imaging experiments.

With this knowledge, a setup could be designed to provide a physiological blood flow to the liver. The final setup consisted of two parts; a liver reservoir in which the liver is submerged in blood and the driving system, consisting of two blood pumps — for pulsatile arterial flow and for continuous venous flow — oxygenators, and sensors. Long lines of tubing separated the reservoir placed in the MRI scanner and the driving system in the control room.

During the perfusion of a porcine liver with blood, the hemodynamic parameters were investigated. Pump settings were tuned to reach physiological flow and pressure values. Compared to reference waveforms, hepatic arterial flow was almost twice as low, while hepatic arterial pressure was over twice as high. Mean portal venous flow and pressure values also deviated from the reference values. The main recommendation for reaching physiological flow and pressure waveforms includes further experimentation with pump settings such as pump speed, systole, and pulsatile flow amplitude.

Liver viability during perfusion with oxygenated blood was assessed by analysing several blood parameters over time. Partial gas pressures and pH levels indicated that the pressure of the oxygen supply was too high. Malfunction of liver homeostasis also occurred, reflected in glucose and electrolyte levels. To keep livers viable for longer using this system, it is recommended to apply a lower gas flow rate and to supply additives such as electrolytes and insulin to the perfusate.

Lastly, the system was operated on a porcine liver during magnetic resonance imaging, during which perfusion scans were performed. Qualitative methods— by investigating liver vasculature after contrast administration — indicated that the liver was not homogeneously perfused. Quantitative methods confirmed this observation by approximating the increase of contrast agent concentration over time in different liver regions.

It is recommended to combine liver viability testing and MRI scans in one perfusion experiment. This will give clearer insights into the influence of certain perfusion parameters on liver viability, including homogeneity of perfusion, hemodynamic parameters, certain perfusate additives, and oxygenation. Although perfusion for four to six hours has not been reached in this thesis, a framework is described for what needs to be optimised — regarding both the use of the system itself and preparing livers for perfusion — before microsphere distribution in radioembolisation can be studied with this system.

Samenvatting

De verdeling van microsferen door de lever tijdens radio-embolisatie kan bestudeerd worden door middel van modaliteiten zoals MRI, met als doel om de doeltreffendheid van deze behandeling van het hepatocellulair carcinoom te verbeteren. Ex-vivo geperfundeerde varkenslevers hebben de voorkeur voor dergelijke studies. Deze geven toegang tot het dynamische gedrag van de levers, waarbij gelijktijdig de input bloedstroom bestuurd kan worden. Er bestaan klinische systemen die de functie van donororganen kunnen behouden tot aan transplantatie. Echter ontbreekt het bij deze systemen aan de controle van enkele perfusieparameters en deze systemen kunnen niet altijd afgestemd worden op medische beeldvormingstechnieken. Het doel van dit onderzoek was dan ook om een te systeem te ontwerpen, ontwikkelen en te implementeren dat in staat is om varkenslevers vier tot zes uur lang functioneel te houden terwijl er MRI scans worden uitgevoerd.

Een systematisch literatuur review is uitgevoerd om inzichten te krijgen in de bestaande geïsoleerde leverperfusie systemen die gebruikt worden in combinatie met medische beeldvormingstechnieken. Deze review resulteerde in een overzicht van de omschrijvingen van de perfusie-opstellingen, fysiologische condities die nodig zijn voor het behouden van de leverfunctie — inclusief parameters zoals bloedstroomsnelheid, druk, het type perfusaat en temperatuur — methodes waarmee leverfunctie wordt getest en experimenten met medische beeldvorming.

Met deze kennis kon een opstelling ontworpen worden om de lever te voorzien van een fysiologische bloedtoevoer. De uiteindelijke opstelling bestond uit twee delen; een lever reservoir waarin de lever werd ondergedompeld in bloed en het besturingssysteem, bestaande uit twee bloedpompen — één voor pulsatiele arteriele bloedtoevoer en één voor continue veneuze bloedtoevoer — oxygenatoren en sensoren. Met behulp van lange slangenlijnen werd het lever reservoir in de MRI scanner gescheiden van het besturingssysteem in de controlekamer.

Tijdens de perfusie van een varkenslever met bloed zijn de hemodynamische parameters onderzocht. De pompinstellingen zijn zo aangepast om fysiologische bloedstroomsnelheden en drukwaardes te bereiken. In vergelijking met de golfvorm van bloedstroom uit referentie literatuur was de hepatische arteriele flow bijna twee keer zo laag, terwijl arteriele bloeddruk meer dan twee keer zo hoog was. De gemiddelde flow en druk waardes in de vena porta weken ook af van de referentiewaardes. De belangrijkste aanbeveling om fysiologische golfvormen voor flow en druk te bereiken is om meer te experimenteren met de instellingen van de pompen, zoals pompsnelheid, systole en pulsatiele flow amplitude.

Gedurende perfusie met zuurstofrijk bloed is de leverfunctie onderzocht door enkele bloedparameters te analyseren. Partiële druk van bloedgassen en pH waardes toonden aan dat de gasdruk van de zuurstoftoevoer te hoog was. Uitval van homeostase in de lever trad ook op, weerspiegeld in de concentraties van glucose en elektrolyten in het bloed. Om levers langer levensvatbaar en functioneel te houden met dit systeem geldt de aanbeveling om een lagere gasdruk toe te passen en om toevoegingen zoals elektrolyten en insuline aan het perfusaat toe te voegen.

Tenslotte zijn MRI scans gemaakt van een varkenslever tijdens perfusie. Kwalitatieve methodes — door de lever vasculatuur te onderzoeken na het toedienen van een contrastmiddel — duidden aan dat de lever niet homogeen doorbloed was. Met kwantitatieve methodes is deze observatie bevestigd door het verschil in de concentratie van contrastvloeistof in verschillende delen van de lever over de tijd te benaderen.

Het is aanbevolen om het testen van de leverfunctie en het uitvoeren van MRI scans te combineren in één perfusie-experiment. Dit zal duidelijkere inzichten geven in de invloed van zekere perfusie-parameters op lever viabiliteit, waaronder homogeniteit van doorbloeding, hemodynamische parameters, enkele toevoegingen in het perfusaat en zuurstoftoevoer. Hoewel leverperfusie gedurende vier tot zes uur niet behaald is in dit onderzoek is er wel omschreven welke zaken verbeterd moeten worden — zowel het systeem zelf als het voorbereiden van de levers voor perfusie — voordat dit systeem gebruikt kan worden voor het bestuderen van de verdeling van microsferen in radio-embolisatie.

Contents

1 Introduction

1.1 Problem definition

Hepatocellular carcinoma (HCC) is the most dominant type of liver cancer, counting for at least 75% of all liver cancer cases.[1,2] In addition to that, HCC is the second most frequent cause of cancer-related deaths globally.[2] With most incidences occurring in Asia and Africa — and case numbers growing fast in European and American countries — it has become a global health issue.[1]

The treatment of hepatocellular carcinoma is complex, as surgical resection is not suitable for all HCC patients[3] and waiting lists for donor organs are often quite long.[4] Several minimally-invasive treatment alternatives have been developed over the past few years, such as selective internal radiation therapies, for example, radioembolisation. In this therapy, microspheres loaded with a radioactive isotope are injected via a catheter into the hepatic artery, carried along by the bloodstream to eventually get lodged in the small blood vessels around the liver tumor.[5,6] There, the beads deliver a high dose of radiation and diminish the blood supply, slowly shrinking the tumor over time.

The microspheres are clearly visible on both single-photon emission computed tomography (SPECT/CT) and magnetic resonance imaging (MRI) scans. These imaging modalities can thus be used to visualise the distribution of microspheres throughout the liver and to quantify the absorbed tumor dose, aiming to improve the efficacy of this treatment.[5,7]

To be able to test the localisation of therapies such as radioembolisation, it is important to have a testing platform that provides a stable testing environment and control of its technical aspects. Phantoms are often used to model human tissues. However, phantoms cannot always mimic the complex and dynamic behaviour and environment of organs like the liver. When isolated animal livers are used instead, their in-vivo behaviour can be used to its advantage. With the use of these organs, one must ideally keep a liver stable for a prolonged duration of time. Therefore, since the start of the 20^{th} century, the ex-vivo perfusion of animal organs has been a topic of research.[8] Using an ex-vivo perfusion platform, parameters such as the blood flow through the organ, the temperature, and the administration of oxygen can be controlled.

With this information, commercially available perfusion platforms have been created for clinical use. One such device is the Liver Assist (XVIVO, Sweden, Göteborg).[9] By perfusing a donor liver using blood or saline solution, the liver can be kept stable before it is transplanted. However, as these systems are currently mainly used on human donor organs in the clinic, the need arises for a system that can be used in an experimental setting so that animal organs such as porcine livers can be used. Additional advantages of experimental in-house perfusion systems include the possibility for tuning of parameters regarding blood flow, as pumps for example can be replaced easily. Such systems are also more flexible regarding testing of liver viability, either using blood tests or by applying medical imaging.

In this thesis, such a perfusion platform will be developed to be used on porcine livers that is compatible for medical imaging. The goal is to be able to perfuse the liver for four to six hours in order to keep the liver stable and functional for the duration of several MRI experiments. This time frame should allow for perfusion without requiring any antibiotics or other additives that would affect the viability testing results. A physiologically relevant environment for the liver should be created during perfusion. Several tests must be performed to assess the functional condition of the liver over time.

While the liver is perfused, the system will be operated during medical imaging. The imaging modality chosen for this thesis is magnetic resonance imaging (MRI). Tuning the system to this modality is challenging, having to safely work around the magnetic field. MRI allows for the measurement of flow of perfusate in the liver during perfusion in 3D. This will give an indication of the viability and liver function during perfusion, validating whether the system is able to provide a proper blood supply to the liver.

1.2 Research questions

Based on the aforementioned project goal, the following main research question is derived;

How should a physiologically relevant ex-vivo perfusion platform be designed and implemented that can keep a liver viable and functional for four to six hours while applying magnetic resonance imaging?

To be able to answer this main question, several sub-questions can be formed to answer along the way:

- What are the functional requirements to be able to use the platform on an imaging modality such as magnetic resonance imaging?
- How should a physiologically relevant environment be created for the perfused liver?
- What system parameters influence flow and pressure driving parameters?
- What viability-based conditions — like oxygen consumption, blood and bile concentrations, output pressure and resistance in the tubing, and the chance of oedema — indicate a viable and functional liver?
- How should the viability and function of the perfused liver be tested?
- How do the viability and function of the liver change over the duration of perfusion?
- How can magnetic resonance imaging be applied to image the flow through the liver during perfusion? And how can such experiments help form a conclusion on the functionality of the liver?

2 Systematic literature review

2.1 Introduction

When testing treatments or medical imaging modalities, there is a preference to test on isolated perfused organs. They give access to the dynamic behaviour of organs — which is challenging to replicate using phantoms — while still providing a testing environment in which technical parameters can be controlled. No testing on patients or living animals is required either and isolated animal organs are easy to handle, especially regarding medical imaging. To perfuse livers during the course of experiments, researchers often use an in-house system. Such a system can easily be modified and tuned to the specific imaging modality that is applied. Commercial systems — such as the Liver Assist (XVIVO, Groningen, the Netherlands)[9] — are used as well, as they are able to keep livers viable for long periods of time. These studies all describe different perfusion setups and system settings that are specific to the experiments performed on the perfused organs. That is why a clear overview of the perfusion systems reported on in literature is required to be able to compare the outcomes of these studies. The perfusion setups provide valuable information for designing our own in-house liver perfusion setup, including any added features for use in MRI. The applied system settings and perfusion parameters present how a physiologically relevant environment for livers should be created. Descriptions of the applied imaging experiments during perfusion give insight into how the system is to be validated in the MRI room. This systematic literature review, therefore, aims to combine the reported findings of isolated liver perfusion systems that are used in combination with medical imaging modalities.

2.2 Materials and methods

2.2.1 Search method

The review was performed following the PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analyses) guidelines.[10] Scopus, PubMed, and Web of Science databases were used to perform the search, using a predefined query. The following query was the result after various searches and consulting an information specialist (query logbook presented in appendix A.1):

(isolated OR "ex vivo" OR "ex situ") AND (organ OR kidney OR liver OR heart) AND (perfus OR "machine perfus*" OR NMP) AND (MRI OR "magnetic resonance imaging" OR photoacoustic* OR "computed tomography" OR ultrasound OR "medical imaging" OR "diagnostic imaging").*

With the arrival of hollow fiber oxygenators, extracorporeal perfusion using membrane oxygenators was taken into the clinic at the start of the 21st century.[11] Only papers published between 2000 and 2022 were included to make sure the included articles are up to date with modern technology. The results from all databases were combined and any duplicate publications were removed using EndNote (version 20.3, Clarivate, London, UK) based on their title, authors, the year of publication, and the journal of publication.

2.2.2 Study selection

Initial study selection and categorisation, based on abstract and title, was independently performed by the authors (M.E. Krommendijk Bsc and Ir. J.L. van der Hoek) using Rayyan Intelligent Systematic Reviewer (Rayyan Systems Inc., Cambridge, Massachusetts, USA). Thereafter, full-text assessment was based on the following criteria;

Inclusion criteria title and abstract screening:

1. Animal
    - Pigs
    - Cows
    - Sheep
    - Dogs
    - Humans
2. Organ
    - Heart perfusion
    - Liver perfusion
    - Kidney perfusion
    - Pancreas perfusion

3. Imaging modality
    - Magnetic resonance imaging
    - Computed tomography
    - Ultrasound imaging
    - Photoacoustic imaging
    - Positron emission tomography
    - X-ray imaging

Exclusion criteria title and abstract screening:

1. Publication type not of interest
    - Reviews
    - Case studies/patient studies
2. Animal not of interest
3. Organ not of interest
4. No isolated ex-vivo perfusion system
    - Isolated hepatic perfusion
    - Renal perfusion
    - Myocardial perfusion
    - Tumor perfusion or contrast uptake
    - No isolated perfusion system used at all
5. Perfusion system not for full organ perfusion
    - Tissue/cell perfusion
    - Limb perfusion
    - Blood vessel perfusion
6. No imaging modality applied

The studies that were included based on title and abstract analysis underwent full-text screening. To reduce the number of papers to be screened for the purpose of this thesis, only studies in which livers are perfused were included in the full-text screening and data extraction. The following exclusion criteria were applied during full-text analysis;

Exclusion criteria full-text screening:

1. No isolated perfusion of whole liver (or otherwise not related to the research question)
2. Description of perfusion system or settings is not detailed enough
3. Perfusion system described in other included paper
4. Imaging experiments are not performed during perfusion (or imaging procedure not described in sufficient detail)

2.2.3 Data extraction

During the full-paper screening, the relevant data were extracted from the included papers. This extraction process was performed by both authors separately and the results were combined.

2.3 Results

2.3.1 Search method and study selection

Figure 1 depicts the results of the study selection in a PRISMA flow chart. The initial search resulted in 2386 papers, and 27 papers remained after removing duplicates and selection based on abstract and title. After full-text screening, 16 papers were included for data extraction.

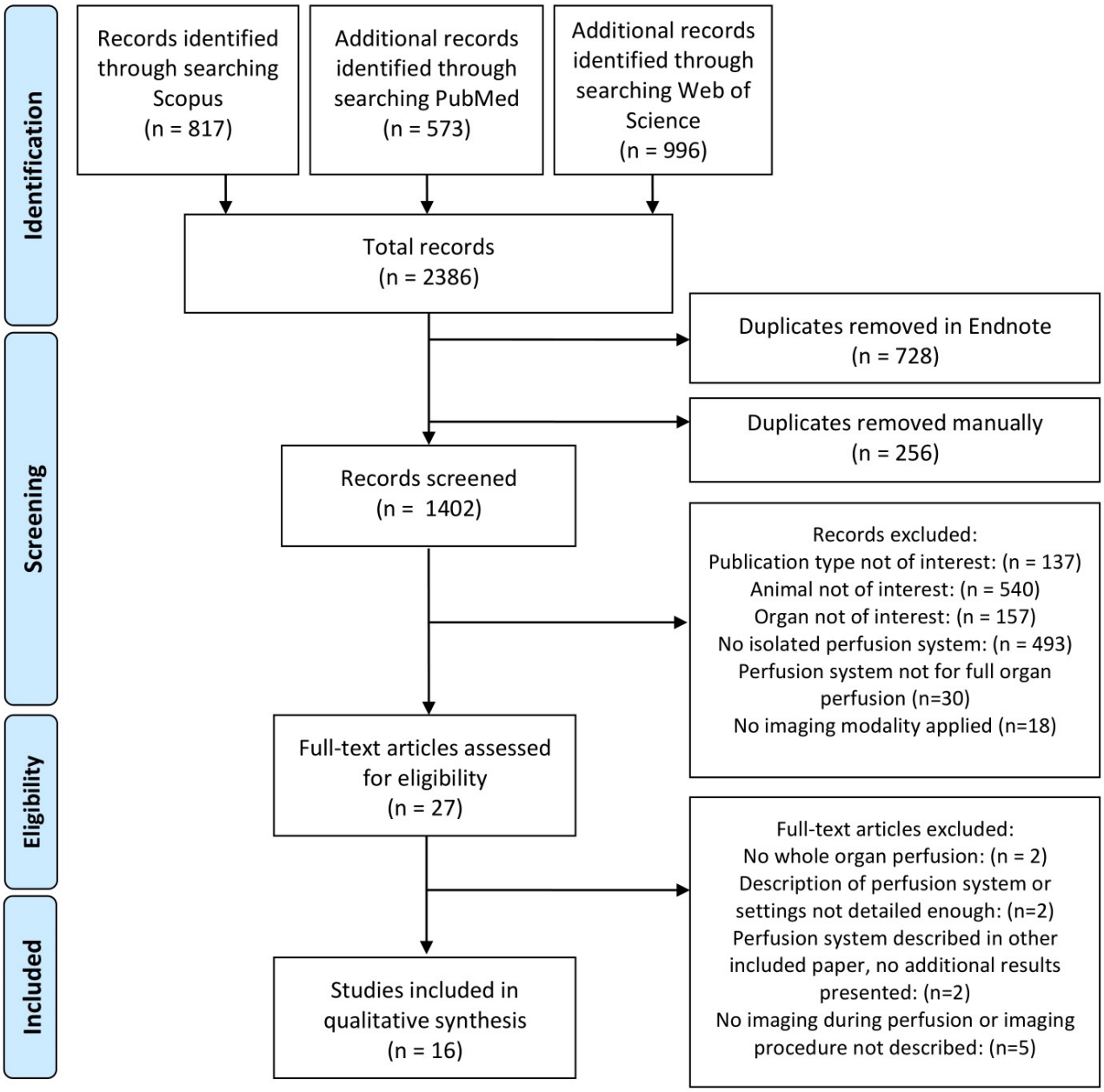


Figure 1: PRISMA chart showing the results of the study selection process. The number of papers included in each step and the reasons for the exclusion of papers are presented in a schematic way.

2.3.2 Data analysis

Perfusate Table A1 in appendix A.2 presents the perfusion details described in each of the included papers. In seven out of 16 included studies, a form of perfusion with blood with additives such as heparin for anti-coagulation, salts, prostacyclins for vasodilation, parental nutrition, or pH-buffers is described. Besides whole blood, four different perfusates have been described that are often used in the field of organ transplantation, such as lactated Ringer's solution,[12] Krebs-Henseleit buffer,[13] Euro-Collins solution,[14,15] or University of Wisconsin solution (also sold under the names of VIASPAN or KPS-1 or kidney perfusion solution[13]).[16] Other papers describe custom-made perfusates, for example containing heparin and various electrolytes.[17]

All preservation solutions described here are electrolyte solutions with certain additives. Ringer's lactate (RL) has an osmolarity comparable to that of blood (275 mOsm/kg), relatively low pH (6.3) has high levels of chloride and sodium ions, and contains lactate.[18] Krebs-Henseleit (KH) buffer is similar to lactated Ringer's solution, but contains low levels of glucose instead of lactate and has a higher osmolarity (312 mOsm/kg).[19] Euro-Collins (EC) solution has an osmolarity of 375 mOsm/kg, contains a high potassium concentration, and had a high concentration of glucose.[18] University of Wisconsin (UW) solution has an osmolarity of 320 mOsm/kg, is also characterised by high potassium levels, and contains insulin and several antibiotics.[18] The comparison of each of these solutions with respect to liver viability will be subject to further discussion in the discussion of this review.

Perfusion temperature Also described in table A1 is the perfusion temperature. Seven papers out of 16 describe a form of sub-normothermic or room temperature perfusion. In some cases, cooling of the organ or perfusate beforehand was described, but no temperature regulation took place during perfusion in these studies. Six papers describe normothermic perfusion, where the organ is actively kept around body temperature. One paper describes hypothermic perfusion and two papers apply both hypothermic and normothermic perfusion in subsequent experiments.

Duration of perfusion Depending on the duration of experiments, livers have been perfused for durations between 30 minutes[20] up to 24 hours,[13,14] as presented in table A1. Whether the organs can stay functional during a long time of perfusion also depends on the preparation steps.

Liver preparation Several different methods of dissection and cannulation have been described in table A1 (only arterial perfusion, only portal perfusion, or both), after which some flushing steps with (often cold) preservation solutions follow to keep livers viable. Total ischemia times ranged from 5 minutes[16] to 24 hours,[21] although warm ischemia —if specified — was limited to a maximum of 30 minutes. Regarding cold ischemia time (CIT), the review has described static cold storage for one hour[15] up to five hours[12] and it is specified that livers can be stored several hours before perfusion if they are submerged in cold perfusate.[22] Static cold storage in such a preservation solution slows down the loss of cellular function.[23] Both warm and cold ischemia and their effect on liver viability will be discussed in further detail in the discussion of this review.

Perfusion setup components Table A2 in appendix A.2 presents an overview of the perfusion setups in the included papers. Different pumps have been described, including the peristaltic Masterflex L/S pump has been used in four of the included perfusion setups[24–27] and has can generate flow rates between 0 and 0.4 L/min.[28] The BioMedicus pumps continuous flow pumps by Medtronic have been used in two of the included studies[12,27] and can reach much higher flows, up to 10 L/min. Regarding oxygenators, various types have been presented in table A2. 11 out of 16 papers presented results of oxygenated perfusion. Several types of oxygenators by Medtronic have been used in some of the publications, intended for flow rates between 1 and 7 L/min.[21,24–26,29]

Once the blood is pumped into the system and oxygenated, it flows into the organ, which is placed in a reservoir. In some reservoirs described in table A2, the outflowing perfusate is collected in this reservoir[15] — keeping the liver submerged in the perfusate — from which perfusate is pumped back into the system. Keeping an organ submerged prevents the liver from drying out, however, blood-air contact will have a negative effect on liver viability if an open reservoir design is used. Some of the bowl-shaped reservoirs contain a double wall filled with water, which is used for temperature regulation.[15] In some cases, a separate perfusate reservoir is used,[14,21] for example, a soft-shell reservoir in the shape of an IV-bag. This requires a much lower perfusate volume and prevents blood-air contact.

Table A2 also presents several sensors for measuring flow rate and pressure. Various types of flow sensors have been described, for example, the variable area flowmeters, in which fluid raises a float in a tapered tube with increasing cross-sectional area.[25,26,29] Rotating vane flowmeters have also been used, which measure the positive volume of water that is displaced between each set of vanes.[21] The pressure sensors described here are medical pressure transducers that make use of a strain-gauge[14] or piezoresistive pressure transducers.[21] As an alternative to these transducers, manometers have also been used to measure pressure.[25,26,29]

Besides the in-house systems described above, existing clinical systems have been reported on in literature as well, as shown in table A2. The extracorporeal circulation unit by Polystan is used by three studies included in this review.[17,22,29] Perfusion systems used in organ transplantation have also been used, for example, XVIVO's LiverAssist system (used by van Leeuwen et al.[16]) and the LifePort system by Organ Recovery Systems (used by Liu et al.[13]).

Hemodynamic parameters The systems described in this review have been used to simulate a broad range of flow rates and pressure values. 13 out of 16 papers have specified boundaries for these hemodynamic parameters. Four of these papers describe flow-controlled setups, four describe pressure-controlled setups and five describe a combination of both set and measured flow rate and pressure values. Table 1 presents the flow rates and pressure values extracted from the included papers.

This table presents a range of values for both parameters, especially regarding flow rate. For providing flow in the hepatic artery, flow rates between 80 and 500 mL/min have been used with a mean applied flow of 271.5 ml/min. For portal vein flow, an even larger range between 6 and 1654.9 mL/min is found with a mean applied flow of 573.3 mL/min. Hepatic arterial pressures ranged between 25 and 110 mmHg (mean 69.3 mmHg). Portal venous pressure ranged between 5 and 123 mmHg (mean 19.8 mmHg) although most papers presented pressure values between 5 and 10 mmHg. The ranges presented in this table are quite large. This will be treated in more detail later in this chapter.

Two papers presented flow rates normalised to liver weight, in units of mL/min/100 g.[15,22] As not every study has presented liver weight (or animal weight), this gives a good presentation of flow in the liver when comparing flow rates from different studies.

	Flow waveform and boundaries		**Pressure boundaries**	
Authors	**Hepatic artery (mL/min)**	**Portal vein (mL/min)**	**Hepatic artery (mmHg)**	**Portal vein (mmHg)**
Alzaraa et al.[29]	210 – 340	730 – 1100	82 – 89	22 – 31
Averkiou et al.[24]	150 – 400 tested, 400 applied during perfusion	400 – 1400 tested, 800 applied during perfusion	44.1 – 73.6*	<7.36*
Bu et al.[22]	300 ± 25**	725 ± 17**	70 – 80 mmHg	8 – 10 mmHg
Civale et al.[14]	500	1500	65 – 110 mmHg	<11 mmHg
Gaffke et al.[15]	18 /100 g (liver weight not specified)	42 /100 g (liver weight not specified)	80	8
Izamis et al.[26]	200 – 300	600 – 900	73.6 – 82.4*	<7.36*
Keravnou et al.[25]	400	800	44.1 – 73.6*	<7.36*
Lorton et al.[30]	_	6 – 9 ***	_	20 – 40
Müller et al.[12]	185 ± 58.6	1482 ± 172.9	_	_
Singh et al.[20]	150	150	_	_
Thompson et al.[27]	80 – 150 tested, 80 applied during perfusion	200 – 1300 tested, 250 applied during perfusion	_	12 – 123 mmHg
van Leeuwen et al.[16]	75-100 for full liver perfusion, 150 – 200 for lateral split perfused liver	200 – 300 (80 – 100 with smaller cannula) for full liver perfusion, >400 for lateral split perfused liver:	25 mmHg	5 mmHg
Zhao et al.[17]	_	100 – 800	70 – 80 mmHg	8 – 10 mmHg
Mean (min – max)	271.5 (80 – 500)****	573.3 (6 – 1654.9)****	69.3 (25 – 110)	19.8 (5 – 123)

Table 1: A table presenting the flow rate and pressure boundaries extracted from the selected publications in this review. The flow rate (in mL/min) and pressure (in mmHg) values are given for both the hepatic artery (HA) and the portal vein (PV).
** Converted from cmH_2O.*
*** Converted from mL/min/100 g with 1850g liver weight.*
**** Converted from mL/s.*
***** Excluding results from Gaffke et al.,[15] since liver weight was not specified.*

Liver viability Only one included paper did not specify any viability testing or conclusions on liver function.[12] All other papers assessed liver viability during and/or after perfusion or derived conclusions from previous/related studies.

Liver viability has been assessed using the following methods:

- Taking histology samples during perfusion, for example, to visualise hepatic architecture endothelial cell structure.[27]
- Investigating hemodynamic parameters were investigated using flow and pressure sensors or using imaging modalities.
- Investigating blood parameters, such as blood pH, glucose levels, and gas contents.
- Testing liver-specific parameters, such as bile production.

The way the analysis of blood gas contents and the measurements liver-specific parameters have been used to assess liver viability are presented in table 2. In this table, the conclusions on liver viability are also presented.

Authors	Gas contents	Additional organ-specific parameters
Alzaraa et al.[29]	Artero-venous differences for oxygen and CO2 (calculated from arterial blood gases) were measured using blood samples; mean O2 = 267 mmHg, CO2 = 8 mmHg	Bile production and hepatic metabolic rate for oxygen values are investigated during perfusion; mean = 42 mmHg ml/g/min
Bu et al.[22] (Zhao et al.[17] describes the same tests)	No gas supplied or uptake measured	Bile production was monitored continuously during perfusion to indicate organ function/viability.
Civale et al.[14]	Partial O2 and CO2 pressures in the blood were maintained between 10-25 kPa (O2) and 4-6 kPa (CO2).	Bile was collected in a separate reservoir, but no results on bile production are presented. Blood parameters such as pH and glucose levels were measured hourly and maintained at desired concentrations (7.35-7.45 pH and 8-15 mmol/l glucose)
Gaffke et al.[15]	Oxygen consumption (difference in O2 saturation between venous and arterial blood) was monitored during perfusion.	Bile secretion was continuously monitored in a separate collection container.
Izamis et al.[26] (Averkiou et al.[24] and Keravnou et al.[25] describe the same tests)	95% O2, 5%CO2 (Tenaris, Bergamo, Italy) supplied. Oxygen consumption was monitored by measuring partial oxygen tension (at the influx of the HA and PV) using an oxygen probe placed in the bubble trap.	Bile production was noted hourly. Perfusate pH was measured half-hourly (Amarell Electronic, Kreuzwertheim, Germany).
Liu et al.[13]	Both perfusates were oxygenated, but no measurements of oxygen saturation or consumption were described.	Transaminase release (Aspartate aminotransferase) is measured from perfusate samples in both sanguineous (samples taken every hour) and acellular perfusion (samples taken every 12 hours for Kidney Perfusion solution and every 30 minutes for Krebs-Kenseleit Bicarbonate solution).
Lorton et al.[30]	According to Buchs et al.[31] , an oxygen flow of 6 L/min was applied. Oxygenation results in a PO2 of 500 mmHg, although no measurements have been described in this study.	No other parameters measured.
Singh et al.[20]	Gas mix of 14% O2, 5% CO2 and 81% N2 supplied to oxygenator, oxygen saturation or other parameters not investigated.	Bile production was monitored continuously to indicate organ function/viability. Blood pH was titrated to a range of 7.35–7.45 by continuously checking pH. If needed, pH was corrected by adding NaHCO3 to the perfusion circuit.
van Leeuwen et al.[16]	Both perfusates were oxygenated, but no measurements of oxygen saturation or consumption were described.	Lactate and pH levels from the perfusate and biliary pH were studied as parameters for liver viability.

Table 2: An overview of the viability tests as described in the reviewed papers. These include measurements of gas contents in the blood and any other liver-specific parameters.

Imaging experiments An overview of the imaging experiments performed in the included papers is presented in table A3 in appendix A.2. Hands-on training of ultrasound imaging skills on isolated perfused livers is common and has been demonstrated on 11 out of 16 of the systems included in this review. For example, the effect of blood flow rate on ablation results by high-intensity focused ultrasound (HIFU) can be investigated.[17,22]

Besides studying outcomes of medical therapies, several included papers have described the use of medical imaging to investigate the extent of perfusion in the organ, either qualitatively or quantitatively. For example, the paper Izamis et al.[26] presents the use of dynamic contrast-enhanced ultrasound imaging (DCEUS) for imaging the micro- and macrovascular structures of the liver. In the study by Müller et al,[12] contrast-enhanced CT scans have been applied to image perfusion in different liver sections in a similar way. From both types of scans, time-intensity curves can be calculated to quantify the amount of contrast that has entered a region of interest over time, giving a quantitative analysis of perfusion. Similar investigations have also been performed using MRI.[21] Contrast-enhanced MRI scans can be used to visualise liver vasculature, and by investigating the intensity of contrast agent in a certain region over time, flow rate can be quantified. This will be explained in more detail in the discussion of this review.

2.4 Discussion and recommendations

2.4.1 Summary of review

This review has resulted in an overview of existing isolated liver perfusion systems reported on in literature. From this overview, it became clear which parts of equipment make up a perfusion system. Different pumps, oxygenators, and reservoir designs compatible for MRI were presented. Different perfusates — such as whole blood, lactated Ringer's solution, and University of Wisconsin solution— were described in this review. Perfusions at various perfusion times and temperatures were presented as well. Table 1 compares the different flow rate and pressure values found in this review, although the provided ranges for these values are broad. Several methods of liver viability testing — such as histology, investigating blood gas parameters, and bile secretion — were also presented. It is important to form our own definition of a viable liver with elements that are important to assess for this system. Lastly, perfusion was combined with medical imaging, most often using ultrasound imaging or MRI. Inspiration for the MRI-validation stage of this thesis has also been drawn from this overview.

2.4.2 Perfusates and oxygenation

Perfusate type In this review, five papers described perfusion with blood. The advantage of using blood over these other perfusates is that blood parameters can be assessed as a measure of liver viability and can easily be compared to reference values. The added benefit of using blood is that the dynamic viscosity of the perfusate remains the same, mimicking the in-vivo flow of blood more closely. This is difficult to mimic with other perfusates. On the other hand, blood requires a more time-consuming method of handling and cleaning. Preservation solutions such as Ringer's lactate are easier to work with and can keep livers viable for several hours as well (under the right conditions).

Studies have compared the different preservation solutions for flushing and preserving livers before transplantation. In a study in dog livers, livers flushed with University of Wisconsin (UW) solution showed less oedema, better glucose metabolism, and better bile production during transplantation than livers flushed with Ringer's lactate.[18] When preserving dog livers in different solutions for 24 hours, the same study showed less liver damage in livers stored in UW solution than in livers stored in Euro-Collins (EC) solution.[18] In a study on rat lungs, the preserving effects of UW have also proven to be much better than those of Krebs-Henseleit buffer, although EC solution provided more uniform protection.[19] By combining such findings, UW has been deemed the most suitable for organ transplantation and (re)perfusion.

However, there are also disadvantages to University of Wisconsin solution. Due to its high viscosity, red blood cells are not washed out fully during the initial flush, although other studies have shown starches in the solution make red blood cells aggregate.[32] One way to work around this issue is to perform an initial washout with a less viscous preservation solution, such as Ringer's lactate or Krebs-Henseleit buffer. An additional disadvantage of both UW and EC solutions is that they contain high levels of potassium, which may lead to vascular endothelial damage.[19] Still, with controlled blood testing, these solutions can be regarded as more effective for preserving and perfusing isolated livers.

Oxygenation One paper was included in the review that describes liver perfusion with non-oxygenated blood. In this paper, it is specified that the liver functioned properly based on bile production and that Doppler Flowmetry results showed proper circulation in microvasculature.[20] Regarding perfusion with a perfusate other than blood, four studies described perfusion without oxygenation. Out of these, two papers specified proper liver function using hemodynamic parameters, histology, and bile production.[17,22] Even though more papers specified viable organs when oxygenation was applied, livers that did not receive any oxygen were still regarded as viable in some studies. Additionally, only one of these studies describes the investigation of lactate levels for assessing liver viability.[16] This is an important parameter to measure since impaired lactate clearance is a clear indicator of cell damage and impaired liver function.[33]

The fact that viability results vary so much between different perfusate types and oxygenation levels could mean one of two things; the papers either have a broad definition of a viable liver, or perfusate oxygenation levels do not have such a large effect on liver viability. Research on the influence of oxygenation shown that pig livers that went oxygenated hypothermic perfusion with diluted blood demonstrated much lower degrees of mitochondrial and nuclear damage than livers that underwent the same perfusion procedure without any oxygen.[34] Oxygen requirement under hypothermic conditions is lower due to the lower metabolic activity, so for normothermic perfusion, oxygenation is even more important to enable full metabolic activity.[35] Additionally, the fact that the included papers are quite broad in their conclusions (see table A4 in appendix A.2), indicates that the first theory applies. It is therefore recommended to form a clear and concise definition of what is considered a viable liver for this project. Blood parameters that relate to perfusate oxygenation and are influenced by perfusate type — such as partial gas pressures, glucose levels, and pH — will be included in this definition.

A variety of oxygenators has been demonstrated in this review. Since fiber material and diameter vary over the types of oxygenators, the priming volumes and levels of compliance — how much the volume in a vessel or fiber changes when pressure is applied — also vary. This means that each oxygenator provides a different resistance to the flow and is suitable for each flow rate. It might be useful to gain more insight into the oxygenation effects of different types of oxygenators on organ viability.[22]

2.4.3 Perfusion temperature

Another parameter that influences liver viability is perfusion temperature. An advantage of hypothermic perfusion is that when cold enough temperatures are applied — between 4 and 8 °C — less oxygen is required, because the low temperature will slow down the organ's metabolism, limiting the demand for oxygen.[36] However, as the metabolism is slowed down, accurate viability testing by assessing blood parameters is not possible. Normothermic machine perfusion mimics physiological conditions of the liver, as it is perfused around body temperature. Additional oxygenation is required, but as the cellular metabolism occurs as normal, liver viability can be tested.[36]

Out of the nine papers that confirmed that livers were viable and functional after perfusion, four describe normothermic perfusion, three sub-normothermic perfusion, one hypothermic perfusion, and one subsequent normothermic and hypothermic perfusion. According to the theory described above, hypothermic perfusion at low temperatures does not require oxygenation. The one paper describing hypothermic perfusion did not supply any oxygen,[17] however, the temperature was not low enough to keep liver metabolism low. Still, the liver was regarded as viable based on hemodynamic parameters, histology, and bile production. The definition of a viable liver applied in this paper might not have been correct, also because blood parameters such as oxygen consumption and lactate have not been investigated. The influence of oxygenation levels on liver viability described in this review will be investigated in more detail.

2.4.4 Warm and cold ischemia before perfusion

The total duration of ischemia ranged from several minutes to 24 hours in the included papers of this review. Several publications note that a warm ischemia time (WIT) of maximum 30 minutes is important for liver viability[22,26] and all papers that specified warm ischemia times stayed within this limit. The shorter a liver is exposed to warm ischemia, the better the outcomes of liver viability will be. However, some time is always required to obtain an organ package, cannulate blood vessels, and start flushing with preservation solutions. A study in rat liver transplants has shown that livers that underwent warm ischemia times over 45 minutes showed more oedema and cell degradation, leading to irreversible injury to the liver graft when transplanted.[37] In livers that underwent 30 minutes of WIT or less, microcirculation could gradually be resumed normally, while this was more difficult when WIT was 45 minutes and even impossible in the case of 60 minutes of WIT. A warm ischemia time of 45 can be regarded as the maximum for functional liver transplantation, but below 30 minutes is optimal for proper liver viability.

This review has also presented a range of cold ischemia times (CIT) between one and five hours, proving especially successful if livers are submerged in cold perfusate.[22] However, studies on survival rates of liver transplant patients have proven that liver quality starts to decrease after 12 hours of static cold storage[38] and normothermic perfusion is unable to recover liver function when they have been stored for over 48 hours.[38] Livers obtained for isolated perfusion should therefore be perfused within 12 hours of cold flushing or storage in a cold solution.

2.4.5 Flow and pressure boundaries

As described before, each study presents perfusion under different flow rates and pressures (see table 1). Since each organ was perfused under different conditions, a broad range of values can be observed from this table.

Since the conditions of each perfusion are so different, it is recommended to delve into literature reporting on physiological flow and pressure values found in clinical settings. These clinical studies could also give information on the waveforms for pulsatile arterial flow, since those are not specified in the publications included in this review. When comparing flow rate and pressure values, it is wise to limit this literature search to one type of study; for example human patient studies (with values obtained from flow-MRI images or other imaging modalities) or in-vivo studies in pigs in which flow and pressure are measured using invasive catheters. From this overview, concise ranges for arterial and venous flow rate and pressure values can be derived. These ranges can be included in our definition of what is considered a viable liver.

Still, it is recommended to perform viability testing at flow rate and pressure levels around these reference ranges to investigate the effects on hemodynamic parameters. This will determine with more certainty whether the definition of a viable liver from this review was indeed too broad, or whether the hemodynamic parameters do not affect liver viability as much.

2.4.6 Definition of a viable liver

Based on the results of this review and the discussion on hemodynamic parameters, oxygenation, and viability testing, the following definition can be formed of what is considered a viable liver while being perfused by our own in-house system.

A liver is regarded as viable when the following properties have been reached during perfusion:

- Homogeneous perfusion of blood throughout the liver tissue. This can be ascertained by performing contrast-enhanced imaging of the liver vasculature, either using MRI[21] or US[26] during perfusion. The contrast agent enters the liver through the branches of the hepatic artery and/or portal vein, after which it should reach the microvasculature. If the liver lobes are homogeneously perfused, equal amounts of contrast agent arrive in the center and near the edges of the lobes.
- Maintenance of arterial and venous flow rates and pressure values [1]
  - Hepatic arterial flow should be around 271.5 mL/min (mean over pulsatile waveform).
  - Portal venous flow should be around 573.3 mL/min.
  - Hepatic arterial pressure should be around 69.3 mmHg (mean over pulsatile waveform).
  - Portal venous pressure should be around 19.8 mmHg.
- Maintenance of liver temperature. Regulation of perfusate temperature around 37 °C for normothermic perfusion is recommended. When the organ stays around body temperature, the liver metabolism will occur as normal, so blood parameters can serve as accurate indicators of liver viability.
- Maintenance of gas exchange and homeostasis. This is reflected in the following blood parameters: [2]
  - Blood pH should be around 7.35 – 7.45.[14]
  - Partial oxygen pressure should be around 267 mmHg.[29]
  - Partial carbon dioxide pressure should be around 8 mmHg.[29]
  - Oxygen levels should be completely saturated.
  - Glucose levels should approach 8 – 15 mmol/L.[14]
- Maintenance of lactate clearance. Blood lactate levels should decrease to concentrations below 1.7 mmol/L[16]
- Maintenance of bile production with a continuous secretion rate of 4.2 $\pm$ 3.4 mL/h.[24–26]
- Absence of oedema. This can be visualised on anatomical MRI scans[15,21] or assessed by weighing the liver during perfusion.

[1] Values are mean values from the overview in table 1. Hemodynamic parameters will be investigated in more detail in chapter 4 of this thesis.

[2] Blood parameters will be investigated in more detail in chapter 5 of this thesis.

2.4.7 Inspiration for system design and testing

This section describes the system features, viability tests, and imaging protocols that would contribute to the development and validation tests of the system built for this project and other systems in the future.

Perfusion system features Three publications in this review have described a self-built system suitable for perfusion in an MRI scanner.[13,15,30] Several features of these systems can be used when designing the MR-compatible system for this thesis.

On average, 7 to 8 meters of tubing is required to reach the MRI scanner from the control room to avoid any disturbance of the magnetic field.[15,30] However, one of the papers described that the driving system can also be placed safely on the 0.5 mT control line around the scanner.[15] It would be of interest for this thesis to investigate the effect of the long tubing on the flow rate that reaches the liver. If it is found that the long tubing would alter the flow drastically — and this difference cannot be overcome by the pumps — such a setup would also be a good option.

Different forms of MR-compatible liver reservoirs are presented in table A2. One of the included studies demonstrates a reservoir in which the liver is placed on a net, hanging over a plexiglass perfusate bath in which the liver is submerged.[21] This plexiglass bath contains a double wall filled with water for temperature regulation. This would be useful for experiments in MRI, given that the temperature regulation in this reservoir can be performed with a separate pump placed in the control room.[21]

Liver viability testing Several methods have been described in the results of this review for assessing viability of the liver during perfusion. In the review, desired blood pH levels (7.35-7.45 pH) and glucose levels (8-15 mmol/l) for viable livers have been presented.[14] Parameters such as perfusate pH and oxygen saturation can also be investigated from these blood samples.[16] Other parameters that will be taken into account during viability testing of the livers perfused by the in-house system are oxygen saturation, partial oxygen pressure, and partial carbon dioxide pressure[29] Besides analysing blood parameters, imaging experiments will also be performed to visualise vasculature of the liver on contrast-enhanced perfusion scans.

MRI experiments As demonstrated in the results of this review, tissue perfusion in isolated perfused organs can be assessed using imaging modalities such as ultrasound imaging or computed tomography. Magnetic resonance imaging can also be used to give qualitative or quantitative conclusions on organ perfusion. One of the included studies presents MRI angiography scans using fast low-angle shot MRI sequences.[15] A gadolinium-based contrast agent is administered and visualises the vasculature of the liver. This will give a qualitative conclusion on perfusion, since the contrast accumulating in small blood vessels represents proper blood supply to a region of interest.

MRI can also be used for quantitative analysis of perfusion.[21] One of the included papers demonstrated organ perfusion in MRI using T2*-echo planar imaging (EPI). A gadolinium-based contrast agent was administered and time-intensity curves were calculated. Comparing the signal intensities over time before and after contrast administration, the amount of contrast agent accumulating in certain regions of interest can be quantified.

Both types of perfusion scans are useful for the MRI experiments in this thesis. The results of these experiments will give conclusions on whether the system can provide proper blood supply to the liver and will assess viability besides the standard blood tests.

2.4.8 Animals other than pigs

14 out of 16 papers study perfusion of pig livers. One paper perfused human donor livers that were unfit for transplantation.[16] One paper perfused the livers of foxhounds.[21] Since pig organs — and especially livers — resemble those of humans closely, the choice to opt for porcine livers is not without reason. For future research, it would be interesting to investigate if any system settings need to be changed based on the animal type that is studied, especially if the goal is to perfuse these organs for a long period of time.

2.4.9 Limitations of the review

Expanding the review to other organs For the purpose of this thesis, the choice was made to focus on liver perfusions. However, this review was started with the goal in mind to review existing perfusion systems for multiple organs, at least including kidney and heart perfusions. It is definitely worth elaborating this review with studies on these organs. This will gain more insight into what system settings are required for each organ and what specific tests to perform to test their viability.

Development of perfusion systems and risk of bias In studies that describe the development of a new organ perfusion system, the authors are often quite positive regarding the functioning of the perfusion system that was used. There might be a certain risk of bias involved in this, which has not been investigated in this review. When this review is to be extended to also include kidney and heart perfusions, it is certainly worth investigating in more detail if the presented conclusions matched the obtained results.

However, some papers do present concrete proof that the study has contributed to the development of organ perfusion systems. This review contains several studies — sometimes from the same research group — that use perfusion systems reported on in previous research. For example, the system described in the paper by Izamis et al.[26] is used in the studies published by Averkiou et al.[24] and Keravnou et al.[25] This indicates that the systems that are built for research purposes get re-used and further developed for different applications.

Completeness of overview Not many papers specify all of the perfusion parameters that are applied. For example, there are several papers that do not specify the boundaries for flow rate and pressure that were either used as settings or measured during perfusion. Two papers describe only the flow rates that are used/measured, three papers do not describe any boundaries regarding flow or pressure. In order to form a proper relation between the perfusion system and the resulting organ viability after perfusion, knowledge about these hemodynamic parameters is crucial. Conclusions about organ viability in these papers should thus be interpreted with caution. Similar caution should be applied to the two studies in which organ viability is not studied at all[12] or in which only histology samples of the liver after fixation were investigated.[27]

Some papers do not clearly describe all of the equipment that is used either. In most cases, a reference has been made to a system used/adapted from previous research (for example Alzaraa et al.[29]) or the details of a clinical system (for example van Leeuwen et al.[16]). This resulted in quite a complete overview of the equipment and system details used in the existing perfusions.

2.5 Conclusions

Using a systematic way of reviewing literature, a selection of papers has been made that focuses on the isolated perfusion of livers combined with medical imaging. An overview of these studies has been made, including descriptions of the perfusion setup used, the mimicked physiological parameters, testing of liver viability, and the performed imaging experiments.

The described studies included in this review presented many in-house systems demonstrated during experiments with different imaging modalities. Clinical systems used for extracorporeal perfusion or perfusion during organ transplantation have also been reported on, along with the system settings applied to keep livers viable. The flow rate and pressure values reached in these setups varied a lot, while most papers still specified that livers were viable and functional after perfusion. As the same observations could be made for perfusate type and oxygenation, it could be concluded that the definition of a viable liver was broad over the included papers.

That is why a definition for liver viability has been proposed that includes the parameters that are important to take account for this particular setup. Several of these parameters, such as hemodynamic parameters and blood parameters, will be investigated in more detail later in this thesis. This will give more concise conclusions on how liver viability should be tested in the final experimental phases and in future experiments.

The results of this review show that it should be feasible to perfuse a porcine liver for several hours using an in-house MR-compatible perfusion system. Interesting topics of research — both for this thesis and for future research — have been recommended. These recommendations will help tune the perfusion system to the purpose of magnetic resonance imaging. Several protocols for viability testing and imaging (both for perfusion imaging and monitoring therapies) have been reported on, which prove valuable for the system validation testing phase of this research.

3 Requirements and design

3.1 Introduction

With a clear overview of the literature on existing perfusion systems, the requirements and design phase can start. This chapter demonstrates the requirements, setup designs, and choices for equipment for this particular perfusion setup.

3.2 Methods

The requirements for this liver perfusion setup have been set using a specific documentation method; the User Requirements Specification (URS) and Design Requirements Specification (DRS).[39] These are two documents that are filled in to create a complete overview of all user- and design-related requirements, demands, and desired features set for the system. Both documents make use of the same numbering system so that each user requirement is coupled to a design requirement. This ensures that each requirement can be met by a clear and concrete design feature or handling. The sections below explain the requirements specified in each document.

Once the requirements are clear, they are translated into a design for the setup. Based on this design, the proper equipment for each element of the setup can be chosen.

3.3 User requirements

The full document is presented in appendix A.3.1. This document specifies requirements related to functionality and use of the system, as well as requirements for the maintenance of the system. Below, several examples are given for user-related requirements given in this document:

1. All materials that come into contact with blood should be biocompatible.
2. To make the system suitable for MRI, the tubing should be long enough for the main system (on a mobile cart) to be placed at a safe distance from the main magnet while the liver itself is in the scanner.
3. The liver reservoir is to be placed in the MRI room and cannot contain any metal-bearing parts.
4. Pulsatile blood flow in the hepatic artery must be mimicked.
5. Continuous blood flow in the portal vein must be mimicked.
6. Flow rate and pressure must be monitored during perfusion.

3.4 Design requirements

The full document is presented in appendix A.3.2. Below, several examples are given on how these requirements are translated into design features and parameters that can be verified:

1. Tubing, oxygenators, and pump heads are made from biocompatible materials.
2. At least 8m of tubing is used to separate the liver reservoir in the MRI from the driving system in the control room
3. The liver reservoir is made from metal-free materials.
4. A pulsatile flow pump is used to mimic hepatic arterial flow. A pump is chosen that can provide pulsatile flow rates up to 1000 mL/min.
5. A continuous flow pump is used to mimic portal venous flow. A pump is chosen that can provide flow rates around 1200-1300 ml/min.
6. Flow rate and pressure are to be monitored during perfusion using flow rate and pressure sensors.

Additional requirements for the performance, use, and maintenance of the system are presented in both documents as well.

3.5 System design

This set of requirements can now be translated into a system design, this process will be demonstrated in this section. A flow chart showing the relevant equipment, energies, and blood flows through the system will be presented. The setup design concept derived from this flow chart is given after that.

3.5.1 Flow chart

Figure 2 demonstrates a liver perfusion system in a schematic overview. It is split into two parts; a periphery part that drives the equipment that operates on the blood flowing through the blood circuit part.

The dual blood supply of the liver is described in this flow chart. The blood that flows out of the liver (placed in the reservoir) gains hydraulic energy through the blood pumps, driven by the consoles. Blood with the desired flow rate enters the oxygenators, which are coupled to a thermal regulator, gas supply, and a supply of additives. These periphery units provide the blood with the desired temperature and concentrations of oxygen, carbon dioxide, and additives such as glucose and heparin. Now, the blood returns to the liver, after which the blood has lost its hydraulic and thermal energy, additives are depleted, and becomes hypoxic and hypercapnic. Going back towards the liver, the cycle of blood flow can start again, creating a closed perfusion loop. Sensors for monitoring flow rate and pressure are additional features added to the periphery, as well as certain additives like heparin and glucose that might be added during perfusion.

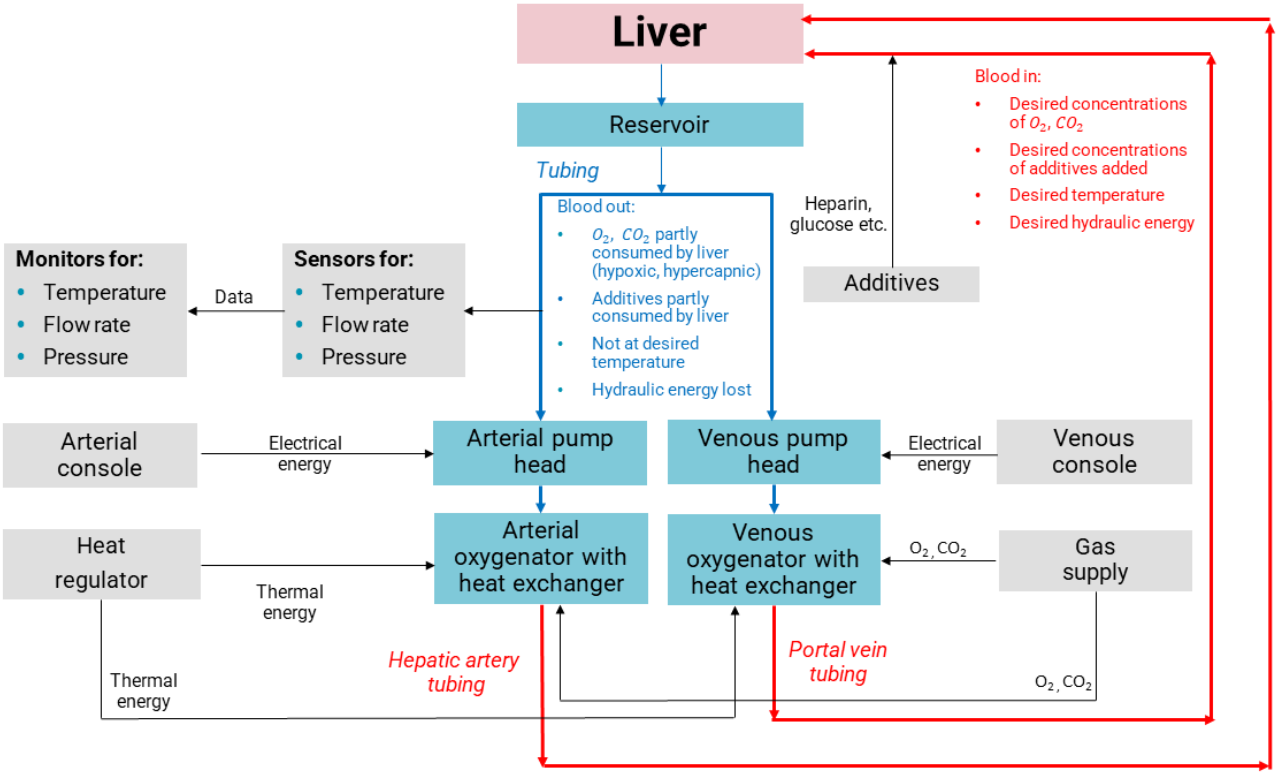


Figure 2: A schematic flow chart describing a liver perfusion system. It shows the required equipment, involved energies and blood flows required for keeping a liver viable

3.5.2 Design concept

Figure 3 shows a schematic design of the setup to be used with blood. The liver is placed in a reservoir, located in the MRI room. By one long line of tubing, it is separated from the driving system in the control room. This line is split into two by a y-connector, heading into the driving system This driving system makes use of two pumps to mimic the dual blood supply of the liver; one arterial pump providing pulsatile flow of blood, and one venous pump providing continuous flow of blood. After that, gas exchange occurs in the two oxygenator (one for each inflow), which are connected to an oxygen supply. The oxygenators used also have integrated heat exchangers. When these are coupled to a heat regulator, the temperature of the blood is also controlled. Then, oxygen-rich blood is pumped along two additional lines of tubing leading back to the liver, closing off the blood flow loop. Sensors are also placed in the setup for monitoring flow rate and pressure. Blood samples for viability testing can be taken from the liver reservoir.

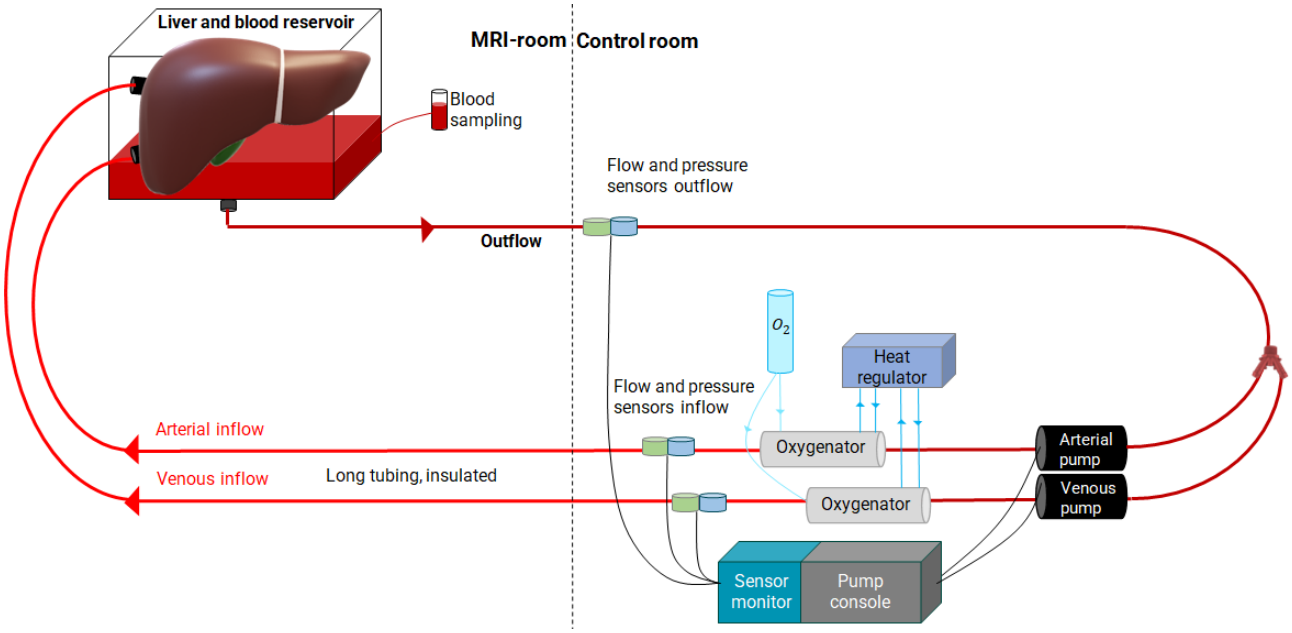


Figure 3: Design concept for the perfusion setup. The blood flow loop consists of a liver in a reservoir, submerged in blood, tubing for blood supply that separates the liver from the driving system, arterial and venous pumps (for pulsatile and continuous flow), and arterial and venous oxygenators. The oxygenators are coupled to an oxygen supply and a thermostat for gas exchange and temperature regulation during perfusion. Additionally, sensors are placed at different points in the system for measuring flow rate and pressure values going in or coming out of the liver. Blood sampling for viability testing can be done either from the reservoir or from one of the lines of tubing.

3.6 Choosing equipment

Now that the first design concept is complete, the most suitable type of equipment for each element must be chosen. This section describes the process of comparing different types of pumps and sensors.

3.6.1 Pumps

Technical specifications Two pumps are required to mimic the dual blood supply of the liver. One pump is required to give a pulsatile blood flow, mimicking that of the hepatic artery. The second pump must provide a relatively continuous flow to mimic that of the portal vein. For these two pumps, several requirements have already been described in this chapter. For example, both pumps must be able to be used with blood. This means that the inner parts (pump heads, membranes, valves, tubes etc.) of the pumps must be able to be cleaned easily. There are also requirements described regarding the output flow.

To compare all of these specifications for each pump considered — both pulsatile and continuous for arterial and venous flow respectively — several specifications are given in table 3. These specifications include pump type, flow range, the range of the mimicked heart rate, connector size (important for connections for tubing), and recommended tubing size (if described). For the pulsatile pumps, it will also be stated whether the output flow profile — and with that, the pulsatility — is programmable or not.

	Pump	Type	Flow range (L/min)	Heart rate (bpm)	Connector size (mm)	Tubing size (mm)	Programmable flow profile
Pulsatile flow pumps	Xenios	Diagonal pump	0.35-2.4 L OR* 0.1-0.8	40-90	6.4 OR 9.5 (1/4" or 3/4")	6.4 OR 9.5	Roughly
	Harvard Apparatus	Positive piston pump	0.005-2 OR** 0.08-6	20-200	Not specified	8 OR** 11 11	Yes
	Triphasic	Digitally controlled positive displacement pump	0.2-1.5 (0-25 mL/stroke)	40-120	6	Not specified, similar to connectors	Yes
	Vivitro	Hydraulic piston pump	0-15 (0-180 mL/stroke)	3-200	19 (3/4")	Minimum 12.5 (1/2")	Yes
	MasterFlex L/S	Peristaltic pump	0-0.4	Not specified	4.8-9.7	4.8-9.7 mm	No
Continuous flow pumps	Maquet Rotaflow	Centrifugal pump	0-10	N/A	9.5 (or 3/4")	Not specified, similar to connectors	N/A
	Harvard Apparatus	Centrifugal pump	0-11	N/A	6.4 OR 9.5 (1/4" or 3/4")	Not specified, similar to connectors	N/A

Table 3: Table of technical specifications for the pulsatile and continuous flow pumps. These specifications include pump type, flow range, the range of the mimicked heart rate, connector size, recommended tubing size, and details about programmability.
** Dependent on oxygenator: pediatric or infant.*
*** Dependent on type: either for rabbits or for dogs and monkeys.*

Advantages and disadvantages Besides these technical specifications, the following more advantages and disadvantages are to be taken into consideration:

- The Xenios pump is blood-compatible, as only the pump heads come into contact with blood. It can also provide a stable pulsatile flow, however, motor control must be modified for programming the flow profile more accurately.
- The ViVitro pump allows for very accurate control of the pulsatile waveform. However, for use with blood, separate pump head parts should be created or printed (or expensive pump head should be ordered), as they are difficult to clean. The same holds for the Triphasic pump.
- The MasterFlex pump is easy to use, however, provides no control of the pulsatility.
- Both Harvard apparatus pumps (pulsatile and continuous) can provide very broad flow ranges, but are very expensive compared to the Maquet continuous pump.

Pump choice Based on the specifications presented in table 3 and the pros and cons presented above, the Xenios pump and the Maquet pump are chosen for pulsatile and continuous flow respectively. Both pumps provide flows in the specified ranges to mimic hepatic arterial and portal venous blood flow. They are also easy to use with blood, as only their pump heads (which are disposable) come into contact with blood. Regarding the pulsatility control of the Xenios pump, for the purpose of this thesis, tests will be done using basic pulsatility control. This will result in pulsatile waves resembling sine-waves. More detailed pulsatile flow control by changing the way the motors are controlled, will be a topic of future research.

3.6.2 Sensors

The chosen pumps given above pump blood around with the flow rate set on the pump console. However, the flow rate in the system differs from this pump setting. The output flow rate of a pumps gets reduced as it meets resistance by the tubing, connectors and other elements in the system. It is therefore important to measure flow rate and pressure values. The same holds for pressure values, since lengths of tubing, connectors and elements like oxygenators greatly influence the fluid pressure in the system. This section describes how the types of sensors were chosen to measure flow rate and pressure values in the system.

Choice flow sensors Many types of sensors exist with which flow rate values can be measured in perfusion systems. For this system, ultrasound-based flow sensors are chosen, since they are capable of providing accurate and real-time flow rate information in tubing systems. Most of these sensors are clamp-on sensors as well, making them easy to use and re-locate in the system if needed. With these benefits in mind, the choice is made between three brands of ultrasound-based flow sensors: Transonic (Transonic Systems Inc, Ithaca New York, USA), Sonotec (Halle, Germany) and em-tec (em-tc GmbH, Finning, Germany).

Each brand has different types of sensors, all compatible with different types of tubing. This also influences the range of flow rates that can be measured with the sensor. That is why, again, a table with technical specifications is made. Table A5 in appendix A.4 shows several different types of sensors by Transonic, Sonotec and em-tec with their respective flow rate ranges and compatible tubing sizes. Besides that, indications of resolution, maximum zero offset, absolute and relative accuracy and repeatability are given as well.

All three sensors have one specific type with a suitable flow rate range. For the Transonic and em-tec sensors, separate interfaces with either the pump or a separate data acquisition card must be made. The Sonoflow sensors by Sonotec come with a program that can be installed to a PC. When connecting the sensor to the PC using a USB, this program can be used to display measured flow and directly save the data. One drawback of the Sonotec sensors is that they are not directly compatible to the blood tubing (which comes in 1/4" or 3/8"). For the purpose of this thesis, the Sonotec CO.56/060 sensor is chosen. This sensor makes use of ultrasound-transit time to measure flow rates up to 6 L/min and is compatible with 5.0 mm (inner diameter) tubing. To use these sensors, smaller pieces of tubing that fit inside the sensor are inserted.

Choice pressure sensors For the pressure sensors, the choice was made for the Honeywell 40PC015G pressure transducer (Honeywell Inc., Freeport, Illinois, USA). This sensor can measure pressure values up to 776 mmHg. Additional specifications are presented in appendix A.4.

3.7 Discussion

3.7.1 Summary of results

This chapter described the requirements set for an isolated liver perfusion platform suitable for use in magnetic resonance imaging. Based on these requirements, the system could be designed and the most suitable equipment could be chosen.

3.7.2 Requirement analysis

All of the system requirements regarding equipment choice and design of the setup have been taken into account in the design shown in figure 3. In the DRS documentation, it is specified whether each has been met for the final setup (highlighted in green), has not been met (highlighted red), or needs further testing (highlighted yellow).

Both the arterial and venous pumps are able to provide the required flow rates, but the flow rates need to be measured during perfusion to validate this. Therefore, the flow rates — along with resulting pressure values — will be measured at different points in the setup and will be compared to reference values. These measurements will be described in the next chapter of this thesis.

An additional requirement that has not yet been fully met is the hemocompatibility of all elements that come into contact with blood. The disposable pump heads and oxygenators are supplied by the same manufacturers of the chosen pumps, are made for safe use with blood in the clinic, and come in sterile packaging. However, the final liver reservoir design (shown in the results of the next chapter) has not been approved for use with blood, as it consists of a plastic tub with connectors for tubing. Additionally, blood-compatible tubing has been used to connect pump heads and oxygenators, however, regular non-coated PVC tubing is used for the lines connecting the reservoir to the driving system. For future experiments — especially when more viability testing is performed — it is recommended to use blood-compatible tubing to rule out any interactions of blood with the plastics. If possible, the reservoir should also be made out of a different material.

The final element that has not been implemented in the final setup is a heat regulator. As was clear from the review, normothermic perfusion mimics the in-vivo most accurately and allows for the most accurate viability testing. It is recommended to use a device such as the Hico Variotherm 550[40] as a thermostat for keeping the temperature around body temperature (37 °C). The final discussion and recommendations of this thesis will discuss this in more detail.

4 Building the ex-vivo perfusion setup and investigating hemodynamic parameters

4.1 Introduction

This chapter presents the method, results, and discussion of the first experimental phase. This phase started off with building the system out of the chosen equipment, preparing a liver for perfusion, and creating a flow of blood or saline solution through the liver. Next, a set of flow rate and pressure measurements during liver perfusion were performed. By measuring these two parameters at different points in the setup, the effect of oxygenators and long lengths of tubing on the hemodynamic parameters was investigated. The resulting values were compared to reference values from literature expected for viable livers.

The hemodynamic parameters are important to measure in order to investigate how the pump settings should be changed to reach these reference values. The oxygenators and tubing are expected to dampen the variation in pulsatile arterial flow — but not mean flow —- as they provide resistance to the blood flow. The fibers in the oxygenators are quite rigid compared to the tubing walls, their compliance is lower, so the oxygenator is expected to cause a larger pressure drop and decrease in pulsatility than the tubing.

These experiments resulted in a set of pump settings — corresponding to the final mimicked flow rate and pressure values — that could be used in the next experimental phases during liver viability testing and MRI experiments.

4.2 Materials and methods

4.2.1 Perfusion setup

Figure 4 below shows the setup built for perfusing porcine livers. A liver is placed in a blood-filled reservoir made from MRI-compatible clear plastic (40L storage container by Iris USA, Surprise, Arizona). To reach the driving system from the reservoir in the MRI and back again, 8m long lines of 7mm ID PVC tubing (Cristal Al water hose by Alfaflex, Mechelen, Belgium) are connected to the reservoir. Blood-compatible (Raumedic, Helmbrechts, Germany) tubing (with an inner diameter of either 1/4" or 3/8") is used to connect tubing to the pump heads and oxygenators.

The Xenios NovaLung iLA ACTIVVE console (Medos Medizintechnik, Stolberg, Germany) with its DP3 pump head generates pulsatile arterial flow. The Maquet Rotaflow pump (Maquet, Rastatt, Germany) with its R-32 pump head provides continuous venous flow. The specifications of both pumps were previously described in table 3. The blood is then pumped through two oxygenators: a square-shaped Quadrox-iD pediatric oxygenator by Maquet on the arterial side — as this shape will cause less dampening of pulsatility — and a cylindrical Hilite 2400 pediatric oxygenator by Xenios on the venous side. When connected to an oxygen supply and a thermostat, both oxygenation and thermal regulation will occur in these oxygenators. Once the liver is perfused, blood flows freely out of the liver from the inferior vena cava. The outflowing blood is collected in the reservoir and can be pumped back into the system, closing the loop.

Also belonging to the driving system are the sensors for measuring flow rate (Sonoflow by Sonotec, Halle, Germany) and pressure (Honeywell, Freeport Illinois, USA). Specifications for these sensors are can be found in appendix A.4. Both sensors are connected to interfaces to a PC. Control software for the Sonotec sensors and MATLAB (MathWorks, Natick, Massachusetts, USA, version 2021b) are used for monitoring, saving, and processing of data from both sensors. As indicated in blue and green, sensors are placed at different locations in the setup, which will be explained in more detail later in this chapter.

4.2.2 Liver dissection and cannulation

Porcine livers were collected from a local slaughterhouse (Slachthuis Haaksbergen), where complete organ packages were obtained from pigs. Within 30 minutes after slaughter (warm ischemia time), the portal vein cannula was inserted. The liver was flushed with 1L of ice-cold NaCl with 25000 I.U. of heparin added, after which flushing with 2L of ice-cold Ringer's lactate followed. The solution could flow out of the liver through the inferior vena cava. After that, all other organs were removed from the organ package and the liver was isolated for transport on ice to the UT. There, a second cannula was placed for arterial flow. This was placed either in the aorta or directly into the hepatic artery itself. When placed in the aorta, small vessels branching off before the hepatic artery were closed off using sutures or clamps, demonstrated in figure 5. When cannulating the hepatic artery directly, a smaller cannula with an inner diameter of $\pm$ 2 mm was used — as it is quite a bit smaller than the aorta — and all other vessels not going into the liver were closed off as well. The cannula placement is demonstrated in figure 6.

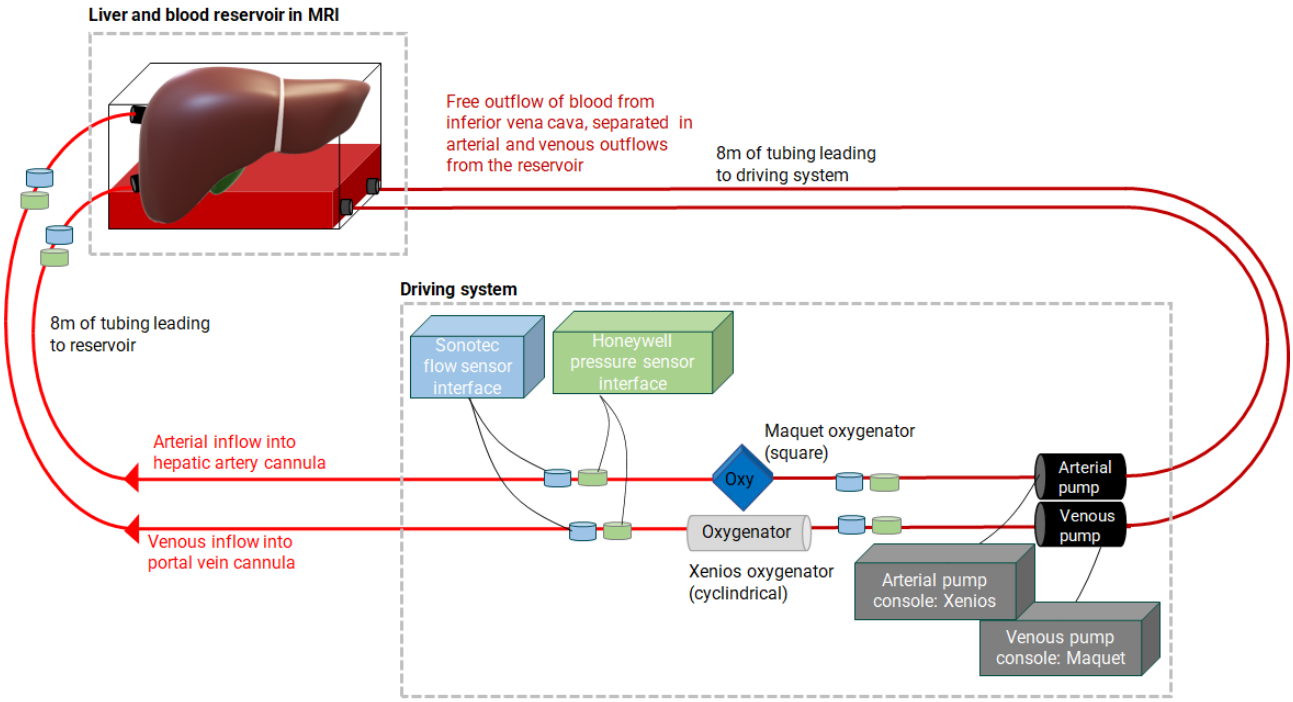


Figure 4: Schematic overview of the perfusion setup. The liver is placed in a reservoir. 8 meters of tubing is connected to this reservoir, which leads blood to an arterial pump (Xenios NovaLung iLA ACTIVVE console with DP3 diagonal pump head) and a venous pump (Maquet Rotaflow with RF-32 pump head). The pumps pump the blood into the oxygenators; the square-shaped oxygenator by Maquet on the arterial side and the cylindrical oxygenator by Xenios on the venous side. The oxygenated blood is pumped further into another 8 meters of tubing, reaching the liver reservoir. Blood enters the liver through the cannulas in the hepatic artery and portal vein. Blood flows out of the liver freely from the inferior vena cava and is collected in the reservoir, closing the blood flow loop. At several locations (indicated in green and blue), sensors are placed for measuring flow rate (Sonotec clamp-on flow sensors) and pressure (Honeywell pressure sensors).

4.2.3 Perfusate

The livers were placed in the reservoir and the arterial and venous cannulas were connected to the arterial and venous lines of the system. 10L of whole porcine blood — pooled from multiple pigs and obtained simultaneously with the organs — was mixed with a solution of additives as a perfusate. This solution consisted of 150.000 I.U. of heparin, 60 mL of NaCl 0.9%, and 18 mL of a 50% glucose and was added to the canister prior to obtaining the blood to prevent coagulation.[41]

4.2.4 Investigating hemodynamic parameters

Sensors For measurements of flow rate, the Sonotec (Sonoflow CO.55 V2.0/060) flow sensor could be clamped directly onto the tubing. Small pieces of 1/8" tubing that fit inside of the sensor were placed in line with the main tubing, as presented in figure 7. These were placed at three measurement locations on both the arterial and venous lines (as indicated by the blue sensors in figure 4). The pressure transducer (40PC015G) by Honeywell was connected to one of the outputs of a three-way valve. This valve could be connected to one of the valves placed at one of the three measurement locations (indicated by the green sensors in figure 4). A small tube filled with water was connected to the valve to transfer the pressure in the main tubing, as presented in figure 8.

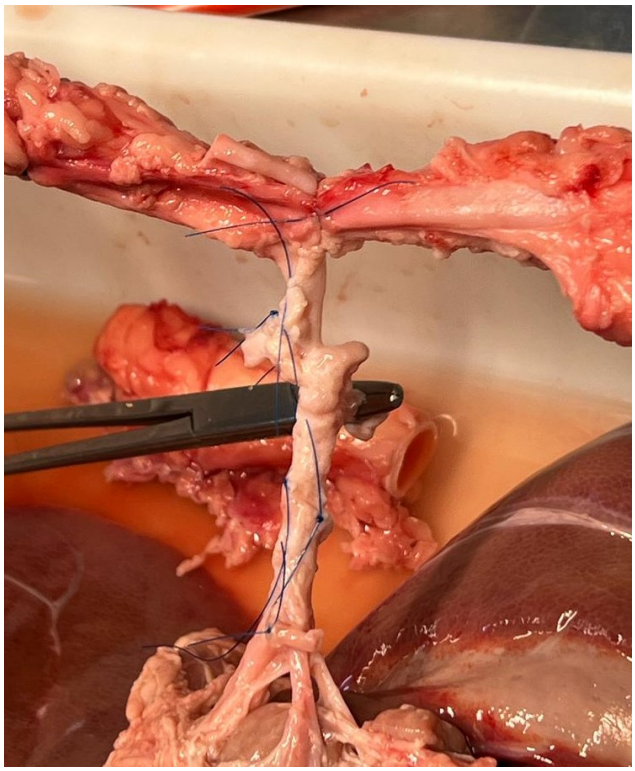


Figure 5: Suturing during liver preparation. Here, many small vessels along the hepatic artery were closed off using sutures (small branches) or clamps (larger branches).

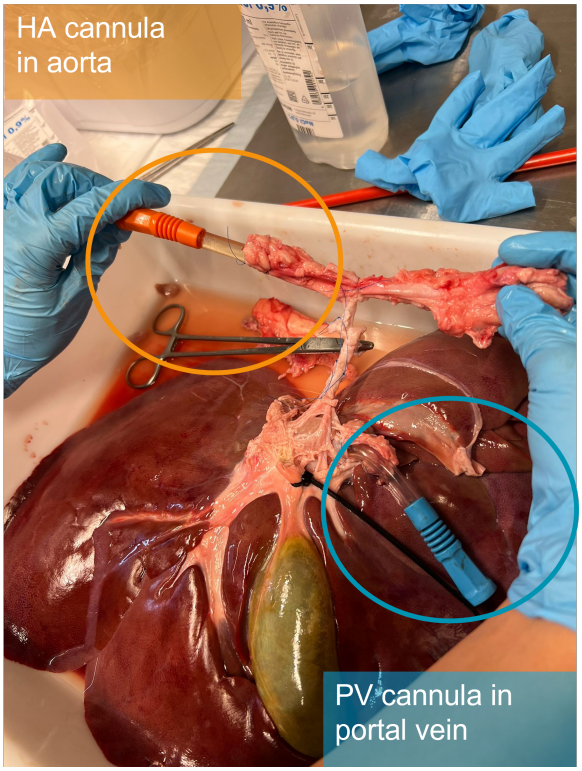


Figure 6: Cannula placement during liver preparation. In orange and blue, the arterial (here placed in the aorta) and the venous cannulas are indicated.

Figure 7: Sonotec flow sensor clamped onto 1/8" tubing for flow measurements.

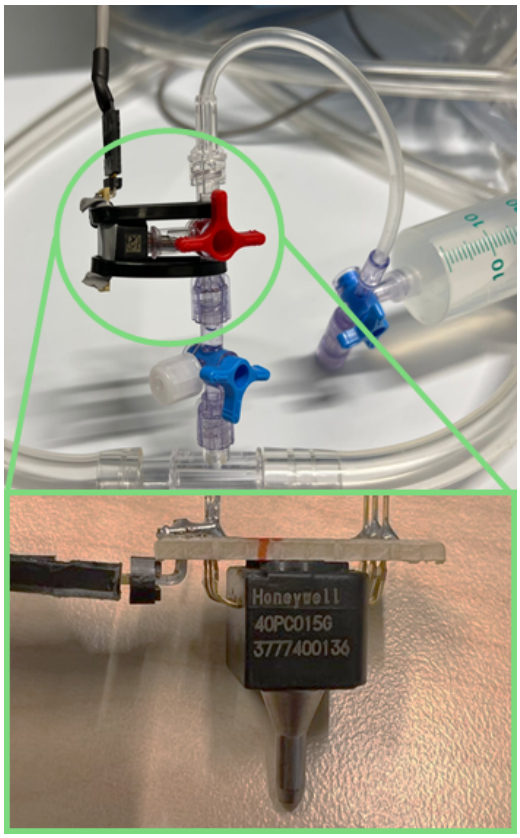


Figure 8: Honeywell pressure transducer attached to a three-way valve for pressure measurements. A small tube filled with water transfers the pressure in the main tubing to the sensor.

Reference values and pump settings The table in appendix A.5 presents reference values for hepatic arterial and portal venous flow rates and pressure values found in literature. These values. clinical values for flow rate and pressure in the hepatic artery, portal vein, and hepatic vein are presented. These values have either been derived from imaging experiments on healthy human subjects or donor livers, or they are based on perfusion models from CT images. From this table, the following ranges were deducted:

- Hepatic artery flow: pulsatile waveform, with a lower limit of 60 mL/min, upper limit of 1000 mL/min, and a mean of 250 mL/min.
- Hepatic artery pressure: pulsatile waveform with a lower limit of 70 mmHg, an upper limit of 100 mmHg, and a mean of 85 mmHg.
- Portal vein flow: 1200 – 1300 mL/min.
- Portal vein pressure: 5 – 10 mmHg.

These pump settings could be reached by changing the following parameters on the Xenios pump:

- rpm: pump speed in rotations per minute.
- Δrpm: change in pump speed over pulsatile waveform, changes how much the amplitude of the waveform changes.
- $pulsefrequency$: pulse repetition rate in beats per minute.
- $systole$: this parameter can be set between 30 and 70 % and will cause a slower or more rapid of acceleration during the first part of the waveform, leading to a subsequent pressure rise.[42]

On the Maquet Rotaflow pump, the rpm was changed to control the pump speed.

Flow rate measurements The pump settings were set to mimic the arterial and venous flow rates mentioned above. The following flow rate measurements were performed using the Sonotec sensors for at least 30 seconds:

- Arterial flow rate after Xenios pump as a baseline measurement with the pump settings as shown above.
- Arterial flow rate after Maquet oxygenator. After this measurement, the pump speed (rpm) or change in pump speed (Δrpm) was increased as needed. This measurement was repeated with the final settings applied.
- Arterial flow rate after 8 meters of tubing, before the arterial cannula. The pump settings are again changed as needed to reach the reference settings. This measurement was repeated with the final settings applied.
- Venous flow rate after Maquet pump as a baseline measurement with the pump speed as shown above.
- Venous flow rate after Xenios oxygenator. After this measurement, the pump speed (rpm) was increased as needed. This measurement was repeated with the final settings applied.
- Venous flow rate after 8 meters of tubing, before the venous cannula. The pump settings are again changed as needed. This measurement was repeated with the final settings applied.

The obtained flow data was saved and loaded into MATLAB. There, the correct time frames were selected and a 2nd order Butterworth filter — with a cutoff frequency at which the magnitude response of the filter is equal to -3dB — was applied to remove noise. For the pulsatile arterial flow, 10 periods were isolated and an ensemble average was taken as the mean waveform. The error over these 10 periods was defined as twice the standard deviation ($2^{*}std$). The upper and lower limits were calculated by adding and subtracting this error from the mean waveform respectively. For the venous waveforms, mean continuous flow rates were calculated based on the mean flow over time.

Pressure measurements The pump settings have now been tuned based on the flow rate and the same settings were applied during pressure measurements. The following resulting pressure values were measured:

- Arterial pressure after Xenios pump as a baseline measurement.
- Arterial pressure after Maquet oxygenator.
- Arterial pressure after 8 meters of tubing, before the arterial cannula.
- Venous pressure after Maquet pump as a baseline measurement.
- Venous pressure after Xenios oxygenator.
- Venous pressure after 8 meters of tubing, before the venous cannula.

The pressure data was saved and loaded into MATLAB. If needed, noise was filtered out using the same Butterworth filter described above. Period isolation and mean waveform calculation were performed for pulsatile arterial pressure data, as well as the calculation of mean continuous venous pressure.

4.3 Results

4.3.1 Final system setup

Figure 9 shows the final liver reservoir used during perfusion. The clear reservoir contains two cable glands to connect the two lines of arterial and venous lines of tubing. From these lines, blood is pumped out of the reservoir. Blood returns to the reservoir through two additional lines of tubing separating the reservoir and the driving system. These lines are directly connected to the arterial and venous liver cannulas and outflowing blood from the liver is collected in the reservoir.

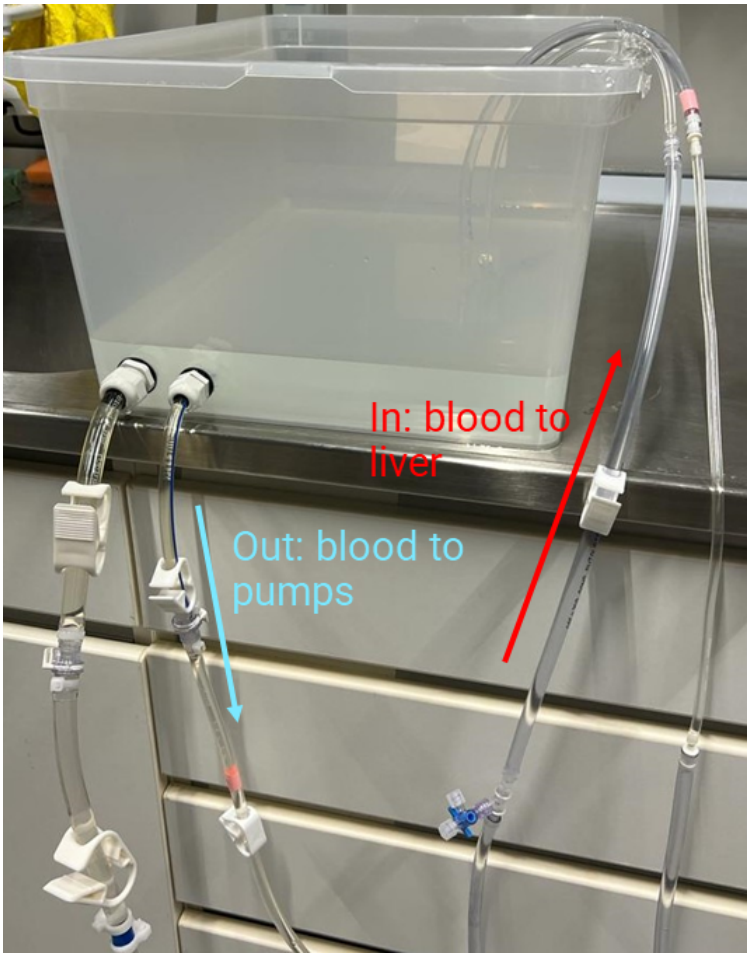


Figure 9: Final liver reservoir. This reservoir consists of a clear plastic tub with connectors for two separate lines of arterial and venous tubing. The driving system pumps this blood out of the reservoir and pumps it through the oxygenators. Oxygenated blood arrives at the liver, where outflowing blood will be collected into the reservoir.

Figures 10, 11 and 12 show the arterial and venous pumps mounted onto the trolley. Both pump heads are connected to the tubing and come in direct contact with blood. On the trolley, the oxygenators can be mounted as well (figures 13 and 14), making the system fully mobile.

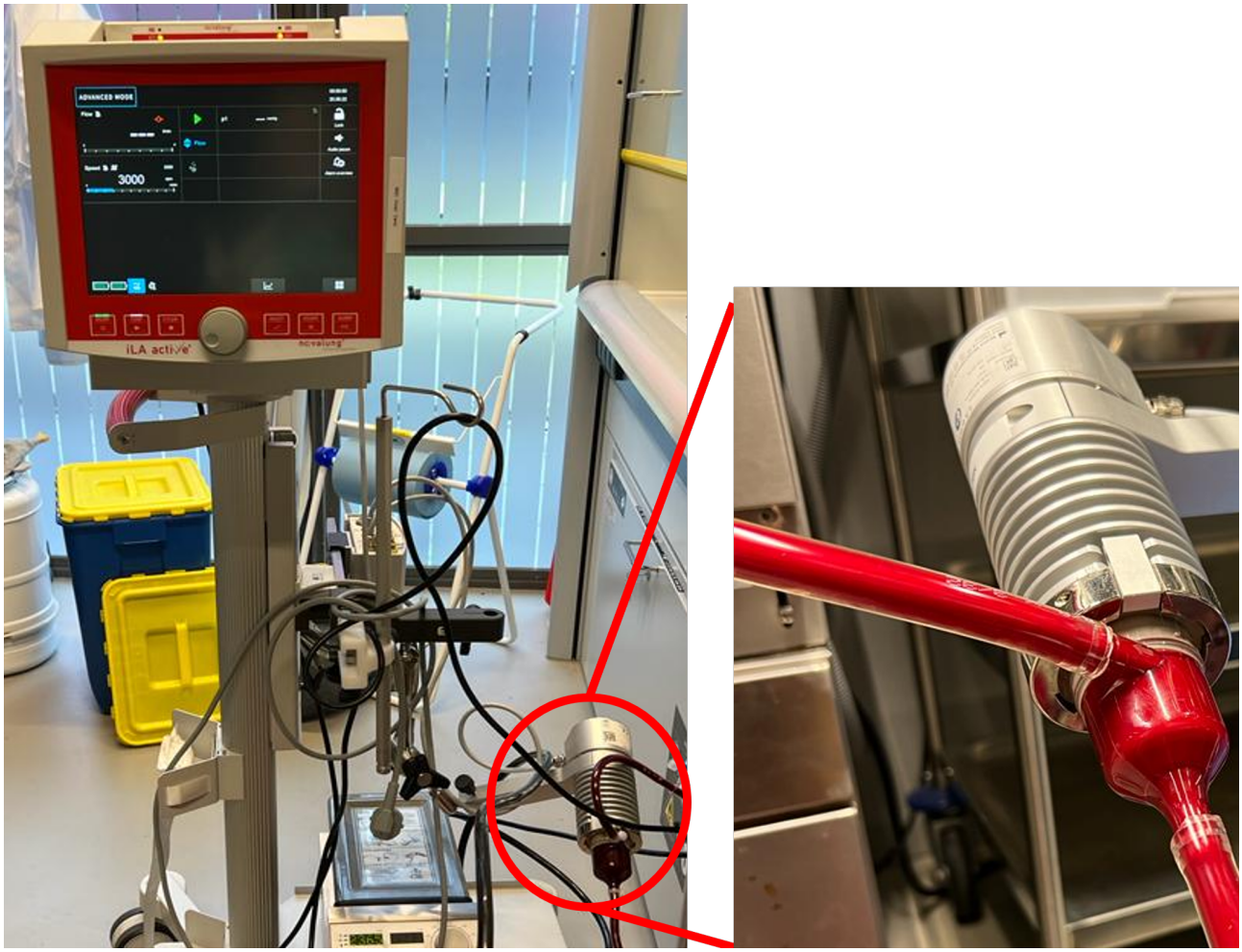


Figure 10: Xenios NovaLung iLA ACTIVVE console for pulsatile arterial flow. The DP3 diagonal pump head (indicated in red) comes in contact with blood and is magnetically connected to the drive, which is controlled by the console

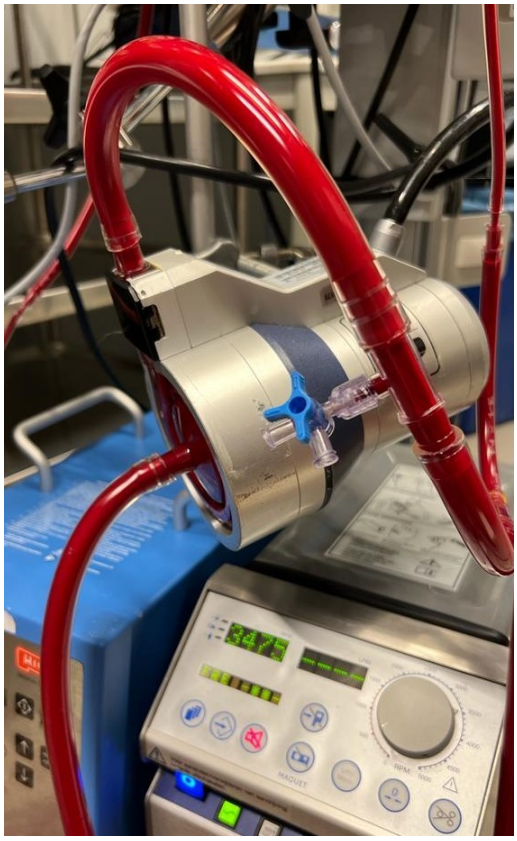


Figure 11: Maquet Rotaflow for continuous venous flow. The RF-32 pump head comes in contact with blood and is magnetically connected to the drive, which is controlled by the console.

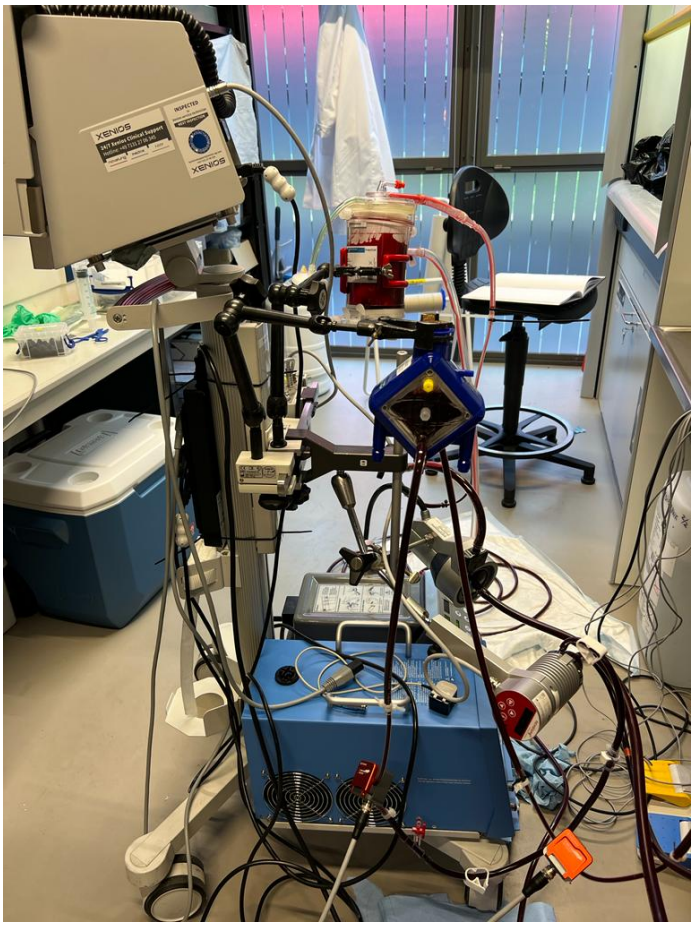


Figure 12: Full setup mounted on the trolley of the Xenios pump.

Figure 13: Maquet oxygenator (Quadrox-iD pediatric) placed on arterial side.

Figure 14: Xenios Hilite 2400 pediatric oxygenator placed on the venous side.

4.3.2 Flow rate measurements

Figure A1 in appendix A.6.1 presents the measurement data of arterial flow rate. Measurements were performed at the following Xenios pump settings, attempting to mimic an arterial flow rate varying between 1 and 16.7 mL/s (60 and 1000 mL/min):

- $rpm = 6000$
- $\Delta rpm = 500$
- $pulsefrequency = 40bpm$
- $systole = 50\%$

The resulting flow that reaches the liver (after oxygenator 8m of tubing, black graph), has become dampened regarding pulsatility. The mean flow stays roughly the same — as there is no loss of blood volume and no change in pump speed. The selected pulse frequency also mimics a lower heart rate than expected. The following pump settings have been chosen to overcome this dampening:

- $rpm = 6900$
- $\Delta rpm = 1500$
- $pulsefrequency = 60bpm$
- $systole = 50\%$

These settings resulted in an increased mean flow, increased pulsatility, and an increased pulse repetition rate. This resulted in the following final waveform (mean waveform and upper and lower limits), presented in figure 15. The maximum flow rate in this waveform — with a mean of 7.5 mL/min — is approximately half of the maximum flow rate of the reference waveform.[43]

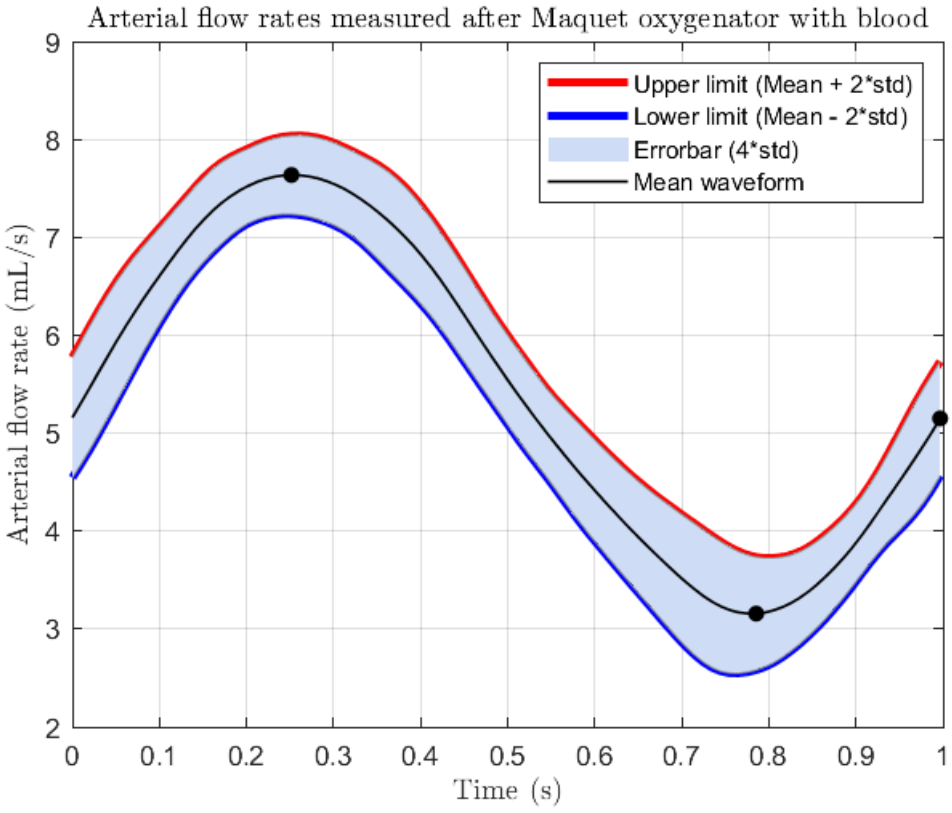


Figure 15: Final arterial flow waveform before liver. The mean waveform and the upper and lower limits varying in the 10 selected periods are plotted.

Figure A2 in appendix A.6.1 presents the venous flow rates measured at different points in the setup at a Maquet pump speed of 3475 rpm, attempting to reach a venous flow rate of 20 – 21.7 mL/s (1200 – 1300 mL/min). The resulting venous flow rate going into the liver ended up being around a factor 1.3 lower than this, 15.87 mL/min. Although this pump provides a continuous flow, the filtered data still shows some variations in flow rate over time. An unexpected increase in venous flow rate after 8 meters of tubing can also be observed. This will be touched upon in the discussion of this chapter.

4.3.3 Pressure measurements

Figures A3 and A4 in appendix A.6.1 show the arterial and venous pressure values measured at different points in the setup respectively, plotted over time. These measurements were performed at the same pump settings described above. The pulsatility of arterial pressure is dampened by both the Maquet oxygenator and the tubing. Mean venous pressure is also reduced by both the Xenios oxygenator and tubing. Both arterial and venous pressure values going into the liver are higher than the reference values; peak arterial pressure (255 mmHg) is over twice as high as the reference value, mean venous pressure (123.1) ended up being around 1.64 times as high as the expected reference.

4.3.4 Influence of oxygenators and tubing

Table 4 presents the minimum, maximum, and mean values of the flow rate and pressure measurements presented above. We can define the amplitude of arterial flow as the difference between the maximum and minimum flow rate. For arterial pressure, we can define the same difference in maximum and minimum pressure as the amplitude of arterial pressure. These two variables quantify the strength of pulsatility, set by the Δrpm setting on the Xenios pump. From this table and figure A1 in appendix A.6.1 can be observed that the amplitude of arterial flow decreases after the oxygenator and the 8 meters of tubing while the mean flow rate stays constant. A similar behaviour is observed for the arterial pressure, having a decreasing amplitude of arterial pressure (see figure **??**). In this case, a drop in mean arterial pressure by both the oxygenator and the tubing can be observed. The Maquet oxygenator causes a decrease in flow pulsatility of around 1.8 mL/s and a drop in mean pressure of 28 mmHg. The tubing decreases the amplitude in arterial flow by 1.3 mL/s and causes a mean pressure drop of approximately 52 mmHg.

Mean venous flow and pressure stayed relatively constant over time— with the exception of the variations presented in figure A2 in appendix A.6.1 — so we look at the mean values of these parameters only. The Xenios oxygenator causes a decrease in flow of approximately 1.1 mL/s and a pressure drop of 111 mmHg. The measurement after the long tubing showed a higher flow rate than the measurements before, However, a pressure drop of 69 mmHg can still be observed.

Both arterial and venous mean flow rates decreased after the oxygenator, however, they increased again after the long lines of tubing. This is not expected to occur — especially seeing as the flow rates were higher than the values measured after the pumps — as tubing walls cause flow resistance. This effect, along with the variation in venous flow rate over time, will be analysed in the discussion of this chapter.

		Before Maquet oxygenator	**After Maquet oxygenator**	**After Maquet oxygenator and 8 meters of tubing**
Arterial flow rate (mL/s)	*Min.*	1.530	2.275	3.081
	Max.	6.350	5.264	4.770
	Amplitude of arterial flow	4.821	2.989	1.689
	Mean	3.872	3.725	3.944
Arterial pressure (mmHg)	*Min.*	171.2	187.3	163.6
	Max.	411.5	334.4	256.0
	Amplitude of arterial pressure	239.8	147.1	92.23
	Mean	288.9	261.2	209.2
		Before Xenios oxygenator	**After Xenios oxygenator**	**After Xenios oxygenator and 8 meters of tubing**
Venous flow rate (mL/s)	*Mean*	15.57	14.46	15.87
Venous pressure (mmHg)	*Mean*	303.3	192.4	123.1

Table 4: Table presenting minimum, maximum, and mean values of the flow rate and pressure measurements.

4.4 Discussion and recommendations

4.4.1 Summary of results

In this experimental phase, the liver perfusion setup was built using the chosen pumps and corresponding oxygenators. Porcine livers obtained from a slaughterhouse were prepared and perfused with blood using this in-house setup while performing measurements of hemodynamic parameters.

The influence of oxygenators and long lines of tubing on blood flow rate and pressure was investigated. In table 4, the boundaries and mean values of these flow and pressure measurements are presented. Pump settings were tuned based on these results. Resulting arterial blood flow going into the liver ended up being almost twice as low as expected from literature[43] and arterial pressure was over twice as high. Resulting venous flow was also lower than the reference, while a much higher pressure buildup was observed. This section will present several clarifications for these differences and will provide recommendations for future experiments.

4.4.2 Comparison to literature

When comparing the mean arterial flow rate and pressure waveforms measured before the liver (figure 16) to waveforms found in literature (figure 17[43]), two main observations can be made: One, the peak pressure is over twice as high as the reference and two, the peak measured flow is almost twice as low as the reference. The low arterial flow rate could have been prevented by setting the pump speed of the Xenios pump higher. However, due to the high pressure build-up, a lower flow rate was applied to avoid failure of the pump. When the saline solution was used during previous experiments (see figure A7 in appendix A.6.4 for the resulting waveform), higher flow rates up to 16 mL/s after the Maquet oxygenator could be reached at a lower pump setting. This indicates that the blood viscosity greatly impacts the flow rate that reaches the liver. When observing venous flow and pressure values in table 4, the mean flow rate of 15.87 mL/min going into the liver is a factor 1.3 lower than the reference flow rate of 1200-1300 mL/min. The mean venous pressure at the height of the liver (123.1 mmHg) is a factor 16.4 times as high as the reference value of 5-10 mmHg.

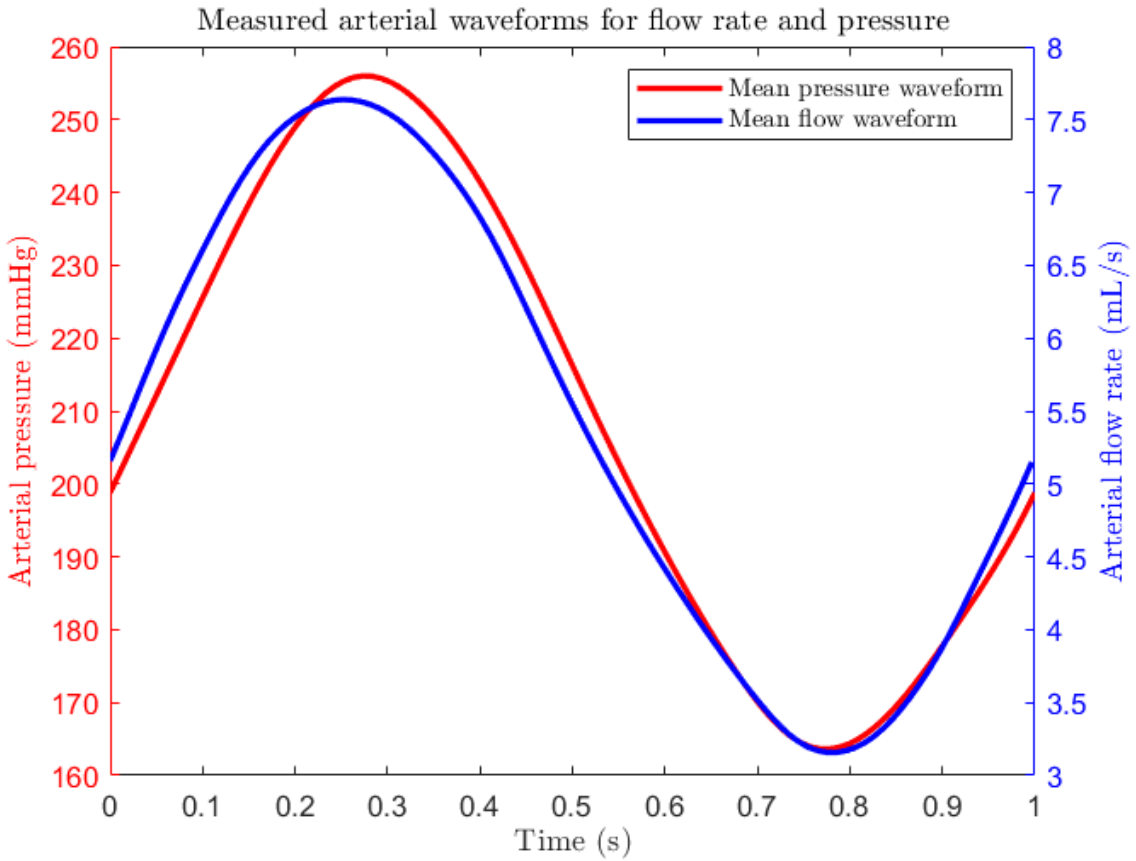


Figure 16: Resulting mean waveforms for pressure (red) and flow rate (blue) measured before the liver.

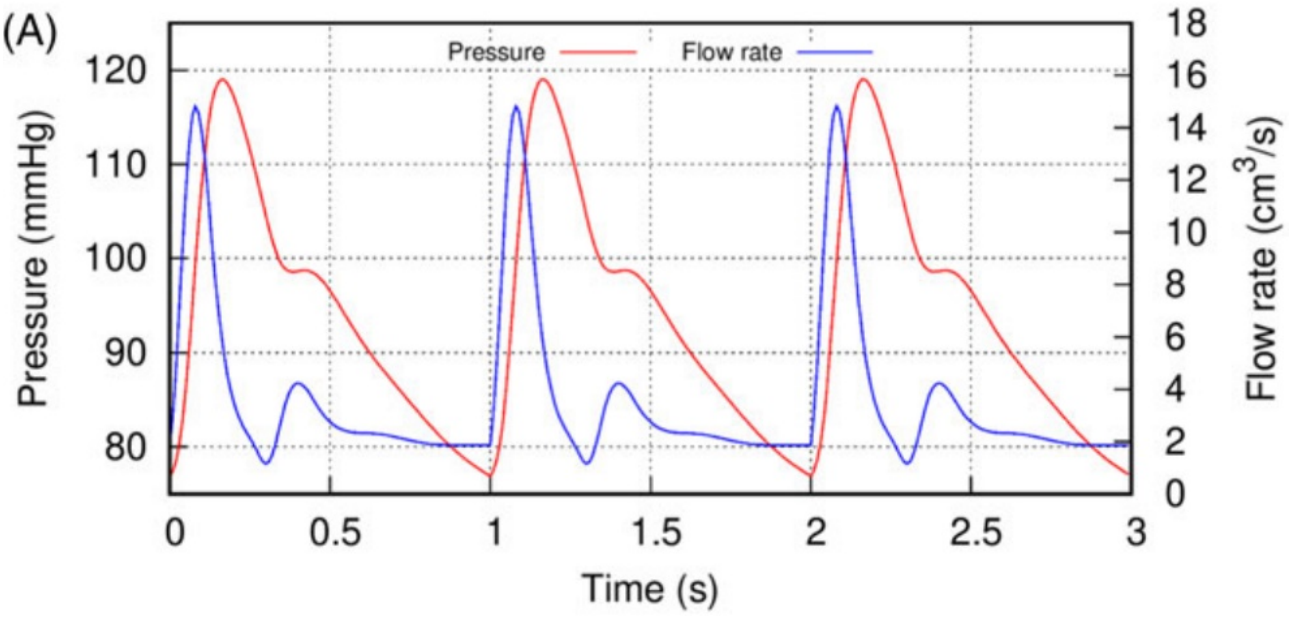


Figure 17: Computed pressure waveforms at the proper hepatic artery (PHA) and input flow rate applied to a simulation.[43] US measurements of proper hepatic artery flow velocities in a liver donor, vessel diameter data obtained from CT data of the same patient, and specific boundary conditions were used to model hepatic artery flow rate and pressure waveforms.

To obtain desired blood flow rates using both pumps, it is recommended to test their limits to approach the reference values closer. By increasing Δrpm on the Xenios pump, the degree of pulsatility can be increased, as has been observed when tuning the Xenios pump settings (noting the difference between the black graph in figure A1 and 15). With that, it is also recommended to use the larger diameter (ID 3/8") blood-compatible tubing by Raumedic. Using a larger tube diameter will cause less flow resistance, leading to a higher flow rate at the same pump speed.[44] As the tubing did not have such a large effect on arterial flow rate as the oxygenators, this might not fully solve the issue, but less resistance is desired.

In the reference flow waveform in figure 17, a signature shape can be observed, presenting the systole and diastole of the heart. The pulsatile flow presented in figure 16 resembles more of a sine wave. To get this waveform closer to the in-vivo reference, the next step would be to investigate the influence of the systole setting on the Xenios pump (%$systole$). For more accurate manipulation of the pulsatile waveform, re-programming the control of the pump drive motor is required.

The waveforms in figure 17 originate were created using a simulation.[43] Ultrasound measurements of proper hepatic arterial flow velocities (in cm/s) in a living liver donor were performed. CT images of the same patient were used to form a model of the vascular tree and vessel diameters were obtained from this. Input flow velocities in the hepatic artery, vessel diameters, output flow velocities in the output (hepatic vein), and a list of boundary conditions were used as input for the model. Using these inputs, this model was able to reconstruct flow rate (in cm^3/s) and pressure waveforms. One thing to take into account when making comparisons with this model is the fact that the waveforms themselves are not directly obtained from patients. The flow is computed from ultrasound measurements, but the pressure waveform is almost entirely simulated, so the simulation might not completely correspond to the in-vivo situation. These waveforms should thus be interpreted with care. In-vivo measurements in (healthy) patients are based on flow velocity, which cannot be compared directly to flow rate measurements over time as performed in the setup. These reference waveforms will approximate flow and pressure in-vivo close enough for now.

4.4.3 Sensor calibration

For both Sonotec flow and Honeywell pressure sensors, the calibration curves are presented in appendix A.6.2 and A.6.3 (see figures A5 and A6). As you can see from the calibration curve, the flow rates measured by the Sonotec values do not match the applied flow rates for the tubing used. The Sonotec flow sensors were calibrated for the small 1/8" blood-compatible tubing and water. Since no calibration has been done for blood, the measurements during perfusion presented here were not calibrated using this curve. The calibration curve suggests that the measurement values are around 1.47 times higher than the actual flow rates, which means that the applied flow rate was even lower in real life.

It is recommended to perform a proper calibration procedure for future experiments. The sensors can be sent to the Sonotec company along with the tubing so that new pre-settings can be made corresponding to the new calibration. This can then be integrated into the Sonotec data processing program used for controlling the sensors and displaying and saving the data. The same method can be applied to calibrate the sensors for blood.

The viscosity of porcine blood can differ quite a lot over time — for blood with 1.0 I.U. of heparin-sodium added, it will decrease from 4.5 to 2.5 $mPa\dot{s}$ over 700 seconds add will increase again after that.[45] It is therefore advised to perform additional calibration measurements to account for these changes. Such measurements would consist of measuring the blood flow rate — at several fixed pump speeds — at different time points after retrieval from the slaughterhouse. This can be done using the Sonotec sensors (once properly calibrated for blood) or a stopwatch and a beaker as described in appendix A.6. For each applied pump speed, an additional calibration curve of measured blood flow over time can be created to take into account during blood perfusion.

4.4.4 Variations in arterial and venous flow

Variations of venous flow rate over time can be observed in figure A2 in appendix A.6.1. The venous flow is not expected to vary over time, since the Maquet pump generates a stable, continuous flow. These variations are most likely due to the way the Sonotec sensors were calibrated for 'zero flow' — a procedure initiated in the control software of the sensors — at different time points during perfusion. The variations in venous flow over time might also be due to sensor placement or movement of the tubing by the strong pulsatility generated by the pump. It is recommended to investigate the effects of this calibration by comparing the measured flow rates before and after performing 'zero flow calibration' several times while making sure the sensors stay in place (preferably laying flat on a table for example).

In figure A2, it can also be observed that the venous flow rate after the eight meters of tubing (black graph) is higher than before (red and blue graphs). This is also observed in the higher mean venous flow of 15.87 ml/s after the tubing and oxygenator in table 4 (compared to the 15.57 mL/s flow rate before the oxygenator). The same effect can be observed for mean arterial flow (3.944 mL/s after the oxygenator and tubing vs. 3.872 mL/s before the oxygenator). The flow rate is expected to decrease due to the resistance the tubing applies to the blood flow, which is the case for the oxygenators themselves (for both arterial and venous mean flow). This effect is most likely also due to the 'zero flow calibration' of the sensors. Once sensors are properly calibrated, it is expected to repeat this measurement during blood perfusion.

4.4.5 Influence of oxygenators and tubing

According to the performance specifications stated in their manuals, the Maquet and Xenios oxygenators should cause a pressure drop of 7 and 10 mmHg respectively at the applied flow rates.[42,46] The measured pressure drops (28 mmHg for Maquet and 111 mmHg for Maquet) are higher than these specifications. As oxygenators were reused several times — and fibers can get clogged up with debris over time — this might have caused an additional resistance.

For one meter 1/4" Raumedic blood-compatible tubing, it is specified to cause a pressure drop of 11 mmHg at a flow rate of 1 L/min as a rule of thumb. Let us assume that the tubing used in this system (also made of PVC, with a similar inner diameter and wall thickness) behaves in a similar way. For 8 meters of tubing, this would mean a pressure drop of around 20 mmHg at a flow rate of 225 mL/min (mean arterial flow of 3.7249 mL/s after oxygenator). The measured arterial pressure drop over the tubing in this setup came in around 52 mmHg. For the venous side, the pressure drop over 8 meters of tubing at a flow rate of 868 ml/min (mean venous flow of 14.4590 mL/s) is expected to be around 77 mmHg. The measured venous pressure drop in this setup was 69 mmHg. The measured values differ slightly from the reference values. This might be due to sensor placement, as the sensors were placed at a greater height for the measurements after the tubing than the measurements at the level of the oxygenators.

Since the pressure drops for both oxygenators and tubing are so different from their expected reference values for both the oxygenators and the tubing, the measurements should be compared with caution. After flow sensors have been properly calibrated and higher flow rates with blood have been reached, it is advised to repeat these measurements with new oxygenators.

4.5 Conclusions

To show how realistically a physiological blood flow to the liver can be established, tests were performed on our in-house system regarding flow rate and pressure. Compared to reference values, both arterial and venous flow rates measured before the liver were low, while pressure buildup due to the oxygenators and long lines of tubing was high. Methods have been presented to obtain accurate flow and pressure values closer to physiological values, such as proper sensor calibration, altering pump speed, pulsatility settings like pulse amplitude and systole, and using new oxygenators to prevent clogging.

The papers included in the systematic literature review presented broad ranges for the applied and measured hemodynamic parameters (see table 1). These values could not be summarised in concise ranges for each parameter, as every measurement was performed under different perfusion conditions. That is why an additional literature search was conducted on physiological reference values measured in human subjects. This resulted in an overview of physiological flow and pressure values for hepatic artery and portal vein (see table A6 in appendix A.5). This literature search also gave more insight into the pulsatile waveforms for arterial flow and pressure, which were not described in the review. Based on these reference values, the statements in the definition for a viable liver that relate hemodynamic flow rates and pressures should be updated as such:

- Maintenance of arterial and venous flow rates and pressure values as follows:
  - Hepatic arterial flow should be pulsatile, with a lower limit of 60 mL/min, an upper limit of 1000 mL/min, and a mean flow of 250 mL/min.
  - Portal venous flow should be around 1200 – 1300 mL/min.
  - Hepatic arterial pressure should be pulsatile, with a lower limit of 70 mmHg, an upper limit of 100 mmHg, and a mean pressure of 85 mmHg.
  - Portal venous pressure should be around 5 – 10 mmHg.

When studying a simulated waveform for arterial flow and pressure[43] instead of mean values, the importance of its signature shape — mimicking the systole and diastole of the heart — became clear. This has not been investigated during this experiment, but the following element should be added to the definition for future research:

- Maintenance of arterial pressure and flow waveforms, mimicking the systole and diastole of the heart.

5 Investigating liver viability

5.1 Introduction

This chapter describes the methods, results and discussion of the second experimental phase. During this phase, the physiologically relevant environment for the liver was mimicked as closely as possible with the equipment on hand. During this phase, a liver was dissected, cannulated, and flushed in such a way that proper liver function is maintained. Regarding perfusion, this included oxygenated perfusion at room temperature while providing the same hemodynamic parameters as described in the previous chapter.

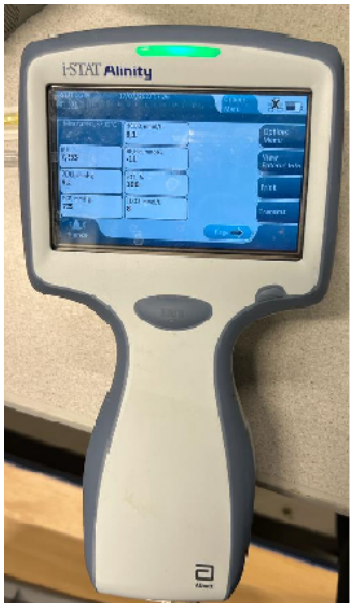
The goal of this experimental phase was to obtain an indication of liver viability by measuring some blood parameters during the course of perfusion. This will verify whether livers can indeed be kept viable for a period of time during experiments using our in-house setup. The results will also indicate if any perfusion characteristics need to change to keep livers viable in future experiments.

5.2 Materials and methods

The liver preparation process, including flushing within the 30 minutes of warm ischemia time — described in the previous chapter — followed by static cold storage, was designed to keep the liver as viable as possible. After dissection, the liver was placed in a bag with Ringer's lactate and placed in an icebox for transport and several hours of static cold storage. Within 5 hours of total ischemia time, oxygenated perfusion at room temperature was initiated. Both warm and cold ischemia times were within the limits investigated in the systematic literature review of this thesis and the limits specified by other studies[37,38]

Perfusion was performed using the same setup as described in the previous chapter. The pumps were set at the same settings and the oxygenators were now connected to an oxygen supply (controlled using a pressure valve set at 1 bar). During liver perfusion with blood — which continued for 2.5 hours — arterial blood samples were taken using a syringe at 3-way valves located near the liver cannulas. Using the i-STAT Alinity device (Abbott Laboratories, Abbott Park Illinois, USA), shown in figure 18. Different blood parameters can be measured, depending on the cartridge that is inserted. A few drops of blood are applied onto the cartridge, after which the cartridge can be closed and inserted into the device.

Before perfusion, activated clotting time was measured in arterial blood using the ACT cartridge (Abbott Laboratories, Abbott Park Illinois, USA). Before perfusion and after 60, 90, 120 and 150 minutes of perfusion, arterial blood samples were taken and measured with the CG8+ cartridges (Abbott Laboratories, Abbott Park Illinois, USA). Figure 18 also shows the CG8+ cartridge filled with blood and presents the list of parameters that is measured using this cartridge.



Sodium (Na)
Potassium (K)
Ionized Calcium (iCa))
Glucose (Glu)
Hematocrit (Hct)
Hemoglobin* (Hgb)
pH
PCO_2
PO_2
TCO_2*
HCO_3*
Base Excess (BE)*
sO_2*

Figure 18: Left: i-STAT Alinity device for blood measurements. Right: CG8+ cartridge for measuring blood parameters. The cartridge is filled with blood and inserted in the device, which will read out values after several minutes. The table presents the parameters that are measured using this cartridge.

5.3 Results

Table 5 below presents the results of the blood parameter measurements over time of perfusion. In the final column, reference values from literature are presented. These values are derived from either pig liver experiments[14,20,47–50] or rat livers.[51]

	Time of perfusion (min)					Reference values from literature
	0	*60*	*90*	*120*	*150*	-
pH	7.227	7.524	7.553	7.555	7.547	7.35 – 7.45[14,20]
PCO_2 **(mmHg)**	69.8	9.1	9.2	9.2	9.1	36 – 45[14,47]
PO_2 **(mmHg)**	40	503	735	784	688	75 – 185[14,47]
HCO_3 **(mmol/L)**	29.1	7.5	8.1	8.2	7.9	22 ± 6[48]
BE, **bld (mmol/L)**	-1	-12	-11	-11	-12	0 ± 2.5[47]
sO_2 **(%)**	64	100	100	100	100	100[49]
TCO_2 **(mmol/L)**	31	8	8	8	8	10 – 40[51]
Na **(mmol/L)**	135	117	118	119	119	148 ± 10[49,50]
K **(mmol/L)**	> 9.0	> 9.0	> 9.0	> 9.0	> 9.0	2.3 – 6.7[47,49,50]
iCa **(mmol/L)**	1.20	0.79	0.79	0.79	0.78	1.13 – 1.3[47]
Glu **(mmol/L)**	13.7	> 38.9	> 38.9	> 38.9	> 38.9	4 – 15[14,47]
Hct **(%PCV)**	43	33	35	33	30	29.7 ± 2.2[50]
Hb **(g/dL)**	14.6	11.2	11.9	11.2	10.2	5.0 – 9.0[49,50]

Table 5: Table presenting the measured blood parameters using the i-STAT with CG8+ cartridges at different timepoints of perfusion. In the final column, reference values from literature are presented.

5.4 Discussion and recommendations

5.4.1 Summary of results

This experiment aimed to investigate liver viability during oxygenated perfusion at room temperature. This was done by taking blood samples over time of perfusion and analysing them using the i-STAT Alinity. With this device, parameters such as pH, partial gas pressures, electrolyte levels, and levels such as glucose, hematocrit, and hemoglobin were investigated (see table 5). These levels were compared to reference values.

5.4.2 Literature regarding reference values

Origin Several papers have been studied to obtain an overview of reference values for the blood parameters Six papers described various blood parameters during pig liver perfusion[14,20,47–50] and one of the papers described CO_2 levels during rat liver perfusion.[51] The reference ranges for pH, calcium, and base excess levels have been described as normal ranges for humans instead.[47] However, whether these correlate with results for porcine livers is unclear.

Stability of values over time The reference values presented are stabilised values that hold for organs that are perfused for a certain amount of time. However long it takes for these values to stabilise differs per study. For example, stability of pH can take somewhere between 0 and 30 minutes, while base excess levels often stabilise within 20 minutes[47] Oxygen saturation is reported to decrease within the first 30-60 minutes, due to an increased oxygen consumption within the first hour of perfusion.[47,49]

The fact that some of these reported parameters reached the desired reference values so quickly — or were within the physiological ranges from the beginning — is dependent on certain additives added to the perfusate. For balancing pH, a buffer in the form of sodium bicarbonate for example has been added in two of the studies.[20,50] An alternative for pH stabilisation is the control of airflow to vary the removal rate of gasses.[14] Glucose levels can be regulated by adding concentrations of glucose and insulin.[14] One of the papers has also described the addition of total parental nutrition, which could help balance electrolytes and glucose levels at the same time.[47]

The sections below present how each of the measured blood parameters compared to the reference values and how they stabilised over time. No additives were used during this experiment to control blood parameters — besides a supply of pure oxygen — so these sections will also describe which additives would be required to reach reference levels in the future.

5.4.3 Partial gas pressures

As can be observed from table 5, PO_2 increased quickly after 1 hour and kept increasing in the next hour. The high partial oxygen pressure resulted in a 100% oxygen saturation within 1 hour. PCO_2 dropped greatly within the first hour of perfusion (especially compared to the reference values) and remained low afterwards. The way these parameters changed over time indicates that the pressure set for oxygenation was too high. When the sweep gas — the gas mixture supplied to the oxygenators, in this case consisting of 100% oxygen — is applied at too high of a flow rate, too much CO_2 is removed.

The resulting decrease in CO_2 levels impacts other parameters as well. In the body, blood pH is regulated by the carbonic acid-bicarbonate buffer system, presented in the chemical equation below.[52] As the total concentration of CO_2 is so low in this case, fewer hydrogen ions get formed, increasing the pH. Fewer bicarbonate ions are formed as well, so the HCO_3 concentration decreases, which results in a lower base excess.

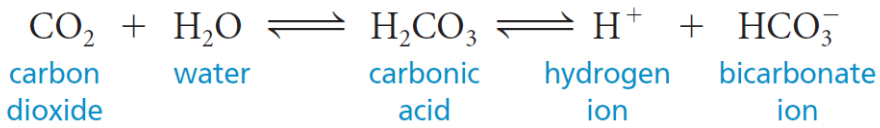


Figure 19: pH regulation through the carbonic acid-bicarbonate buffer system.[52] *CO_2 dissolves in water and is converted to carbonic acid. When blood pH is too low, this acid dissociates into bicarbonate ions and hydrogen ions. When blood pH is too high, the reverse reaction occurs.*

It is recommended to repeat these measurements with lower oxygen pressures applied. It would also be an option to add CO_2 to the sweep gas to increase its partial pressure — for example 95% O_2 and 5% CO_2.[26] Another option would be to only use oxygen but supplied at a much lower sweep gas flow rate. For example, start with an oxygen flow slightly lower than the blood flow rate and decrease this rate — with a flow-regulated valve instead of a pressure-regulated one — to the highest possible flow that will still cause a 100% oxygen saturation. If the pH is still too high after a low sweep gas flow rate is applied, sodium bicarbonate or a similar pH buffer can be used to correct the pH during the course of perfusion.[20,50]

5.4.4 Electrolyte levels

Sodium and ionized calcium levels were high before perfusion and decreased and stabilized during perfusion. Both levels dropped below their specified reference values within one hour of perfusion. Potassium levels were high during the entire experiment and exceeded the measurement threshold.

All three of these electrolytes levels diverging from reference values indicate that homeostasis — keeping electrolyte levels balanced by transport through ion channels — in the liver did not function properly. If these values keep diverging in future experiments, it is recommended to add an electrolyte solution or another form of nutrition during perfusion to keep these levels stable.[47] Since high levels of sodium also trigger oedema,[53] it is also advised to weigh the liver at different timepoints during perfusion to keep track of water retention.

5.4.5 Glucose level

During this experiment, glucose levels increased after one hour of perfusion to levels above the measurement threshold of the device. Since only 18 mL of glucose 50% solution was added to the blood at the slaughterhouse, it is likely that the liver converted some of its stored glycogen to glucose and secreted it into the blood. This indicates again that homeostasis in the liver did not function properly. In future iterations of this experiment, insulin can be administered to regulate glucose levels.[14] A rise in glucose concentration within the first hour indicates a changed glycogen metabolism, which is related to postischemic injury.[49] If similar results are obtained in future experiments, it is recommended to instigate histology samples. These samples could also be used to check cell damage due to a high level of potassium.

5.4.6 Erythrocytes

Hemoglobin levels were higher than the expected reference values, decreased within the first hour and dropped again in the last half hour. The same holds for the hematocrit levels. This indicates that erythrocytes have sedimented to the bottom of the reservoir. It is recommended to stir the reservoir during future perfusion experiments to prevent this sedimentation. This is especially important for large volumes of blood, when big reservoirs are used such as this one.

5.4.7 Other recommendations for future measurements

Several parameters changed greatly within the first hour. For example, the oxygen saturation increased to 100% within the first hour. However, this increase will most likely have been gradual and is not visible from this data. It is recommended to measure more often within the first hour of perfusion, for example, every 15 minutes. This will give a more detailed description of how these values change over time of perfusion.

One parameter mentioned in the systematic review is lactate clearance in blood. This parameter gives a clear indication of how oxygen is consumed by the liver and if liver function is still maintained during perfusion. This parameter can be measured using the CG4+ cartridge, which was not available during this experiment. It is recommended to use this type of cartridge if this experiment is repeated.

5.5 Conclusions

This chapter has shown initial measurements of blood parameters to obtain an indication of liver viability during oxygenated perfusion. These conditions indicated that the perfused liver was not viable during the 2.5 hours of perfusion. However, these results gave insight into changes that needed to be made for future experiments, which include changing the composition and flow rate of the sweep gas for proper gas exchange, adding a buffer to stabilise pH, adding insulin and electrolyte solutions to balance glucose and electrolyte levels, and stirring the reservoir to prevent erythrocytes from sedimenting.

In the systematic literature review, a variety of measured blood parameters such as pH, partial gas pressures, glucose, and lactate[14,20] was presented. Physiological reference values for these parameters were already included in the definition of a viable liver. However, not all blood parameters measured during this experiment were described in the review, so an additional literature search was conducted. This completed the overview of physiological reference values that indicate a viable liver (see last column of table 5). Based on these reference values, the statements in the definition for a viable liver that relate to blood parameters should be updated as such:

- Maintenance of gas exchange levels, reflected in the following blood parameters:
  - Blood pH should be around 7.35 – 7.45.[14,20]
  - Partial oxygen pressure should be around 75 – 185 mmHg.[14,47]
  - Partial carbon dioxide pressure should be around 36 – 45 mmHg.[14,47]
  - Bicarbonate levels should be around 16 – 28 mmol/L.[48]
  - Base excess levels should be around -2.5 – +2.5 mmol/L.[47]
  - Oxygen levels should be completely (100%) saturated.[49]
  - Total carbon dioxide concentration should approach 10 – 40 mmol/L.[51]
- Maintenance of homeostasis, reflected in blood electrolyte and glucose levels.
  - Sodium levels should approach 138 – 158 mmol/L.[49,50]
  - Potassium levels should approach 2.3 – 6.7 mmol/L.[47,49,50]
  - Calcium levels should approach 1.13 – 1.3 mmol/L.[47]
  - Glucose levels should approach 4 – 15 mmol/L.[14,47]
- Maintenance of blood hemoglobin levels in between 27.5 – 31.9 %PCV.[50]
- Maintenance of blood hematocrit levels between 5.0 – 9.0 g/dL.[49,50]
- Maintenance of lactate clearance. Blood lactate levels should decrease to concentrations below 1.7 mmol/L.[16]

6 MRI experiments

6.1 Introduction

The goal of the final experimental phase is to operate the ex-vivo perfusion system while performing MR imaging. This imaging modality allows for the analysis of liver anatomy with a good spatial resolution and good soft-tissue contrast.[21] Besides this, functional parameters such as flow in liver vasculature can be investigated, giving both qualitative and quantitative conclusions on liver perfusion. In the systematic literature review, several MRI experiments for the investigation of liver perfusion have already been described.

In this experimental phase, the vasculature of the perfused liver will be visualised using contrast-enhanced MRI scans, after which both qualitative and quantitative conclusions on perfusion can be drawn. This chapter will demonstrate the MRI sequences and methods of the MRI experiments, as well as the resulting MRI images. The imaging results will be discussed and a conclusion is given on the suitability of the system for liver perfusion during magnetic resonance imaging.

6.2 Materials and methods

6.2.1 MRI sequences and imaging process

MRI scans were made using the Siemens MAGNETOM Aera 1.5T scanner (Siemens, Munich, Germany) present at the UT. The following MRI scans were performed consecutively using different imaging sequences (specific settings are given in appendix A.7):

- $T1$-weighted and $T2$-weighted scans were performed using spin echo and turbo spin echo sequences respectively for imaging the liver anatomy. On $T1$-weighted images, water shows up dark and fatty tissue shows up bright, so there is a large contrast between blood in the vasculature and the surrounding vessels. On $T2$-weighted images, water and fat both show up bright, visualising the liver anatomy very well. These differences are due to timing in radiofrequency pulses during scanning.

- A dynamic fast low-angle shot (FLASH) sequence with a short repetition time was used for angiography scans. This entails scanning the volume several times over time (at approximately 4.2 frames per second for saline perfusion and 1.4 frames per second for blood perfusion) while a contrast agent is administered. This was done using 2 mL of the gadolinium-based contrast agent Dotarem (Gadoteric acid or Gadoterate meglumine by Guerbet, Villepinte, France) at a concentration of 0.5 mmol/mL.

  Of each scanned volume, a maximum intensity projection (MIP) reconstruction was performed. In this process, the voxel with the highest attenuation value is projected on every view throughout the volume onto a 2D image.[54] The pixel-values of the first scan are subtracted from the other scans so that relative contrast is displayed. The 2D images of each slice over time visualise how the contrast agent enters the liver vasculature.

- Dynamic $T2^*$-weighted scans were performed to quantify perfusion. As described in the systematic literature review, by performing echo planar imaging using dynamic $T2^*$-weighted scans during contrast injection, tissue perfusion can be quantified.[21] The liver was scanned over time (at approximately 1.6 frames per second) using this sequence during injection of 1 mL of Dotarem. One liver slice over time was selected and the 100 scans over time were loaded into MATLAB (MathWorks, Natick, Massachusetts, USA, version 2021b). Regions of interest were selected and of each region, a scaled tissue perfusion factor ($STPF$) was calculated based on the mean signal intensity in each region $S(t)$ and the signal intensity before contrast administration S_0. For this, equation 1 below was used and the derivation of this equation is given in appendix A.8.

$$STPF(t) = -\ln\left(\frac{S(t)}{S_0}\right) \tag{1}$$

  The scaled tissue perfusion factors were plotted over time to create time-intensity curves. For different regions of interest in the liver, these curves were compared for relative perfusion quantification.

  Tissue perfusion was first quantified in the four liver lobes to approximate how homogeneously the liver is perfused. After that, several regions within one of the liver lobes were selected to quantify perfusion in regions that look less well perfused than others on the contrast-enhanced scan. For each region of interest, the mean signal intensity was calculated for each scan in time. When these values were plotted over time, the arrival of contrast agent could be observed by a decrease in signal intensity, due to the increased $T2^*$ relaxation rate ($R2^*$).

Signal intensities were converted to scaled tissue perfusion factors ($STPF$, see equation 1) and time intensity curves were made by plotting this factor over time. When the signal intensity of the first time-point was selected for S_0, negative $STPF$ values were obtained after approximately 120 seconds. This indicates that some contrast agent accumulated in the liver during a contrast-enhanced scan previous to this one. To avoid these negative values, the maximum signal intensity from all time points was chosen for S_0 instead. This scaled the graph to the signal intensity measured when all of the contrast agent administered during this scan has washed out.

- A $T1$-weighted scan (using the same sequence as the anatomical one) performed after administration of contrast agent was performed to show a higher-resolution image of the liver vasculature and surrounding tissues.

For the contrast-enhanced scans, there were 15 – 20 seconds of waiting time between the injection of the contrast agent and the start of the scan. This time was based on the time it took for the contrast to reach the liver — dependent on flow velocity and tube length — combined with the scan duration. During this time, the contrast agent was pumped through the tubing and reached the liver several seconds after the scan was started.

6.2.2 Setup and perfusion

During the first iteration of this experiment, a porcine liver was perfused using saline solution. The portal vein cannula was placed according to figure 6 and the arterial cannula was placed in the hepatic artery directly. The pump settings and resulting flow waveform are presented in appendix A.9. In the second iteration of this experiment, a liver was perfused with blood. This time, a large arterial cannula was placed in the aorta, like in figure 6. The same setup and similar pump settings were used as the ones during the investigation of hemodynamic parameters in chapter 4.

The livers were fully submerged in the perfusate in both experiments to improve image quality. The driving system was placed in the control room of the MRI. The long lines of tubing were fed through holes in the wall between the MRI scanner room and the control room (see figure 20), after which perfusion and imaging were initiated.

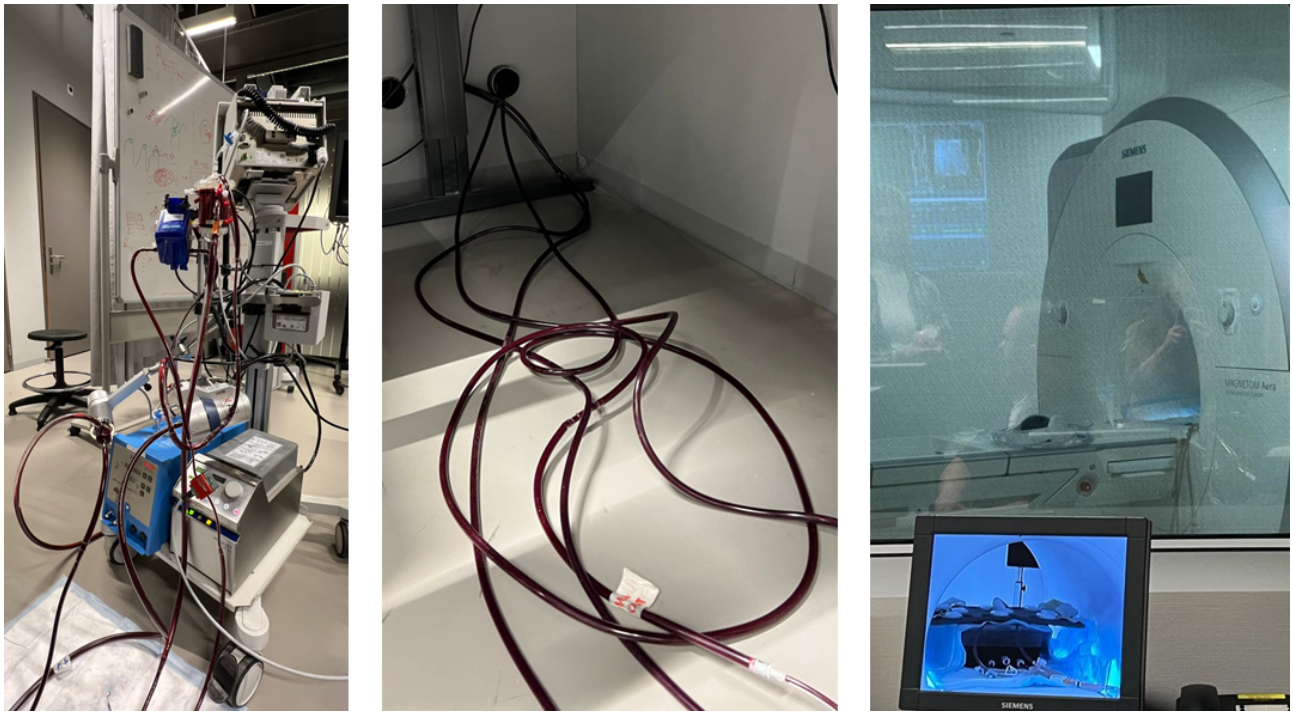


Figure 20: Setup for MRI experiments. The driving system is placed in the control room of the MRI. The 8m long lines of tubing were fed through the holes in the wall. The tubing was connected to the liver reservoir placed in the MRI scanner.

6.3 Results

6.3.1 Saline solution perfusion

Liver anatomy Figures 21 and 22 show the *T*1-weighted and *T*2-weighted anatomical scans of the liver perfused with saline solution. The images show a cross-sectional slice of the liver in the reservoir, viewed from the top. The anatomy of the pig liver with its four lobes is clearly visible, along with the coarse vascular structure of the hepatic artery branches (annotated by the red arrows) and portal vein branches (annotated by the blue arrow). These branch out into smaller vessels, especially well visible on *T*2 (annotated by the yellow arrow). The gallbladder can also be seen between lobes 2 and 3 (annotated by the green arrow).

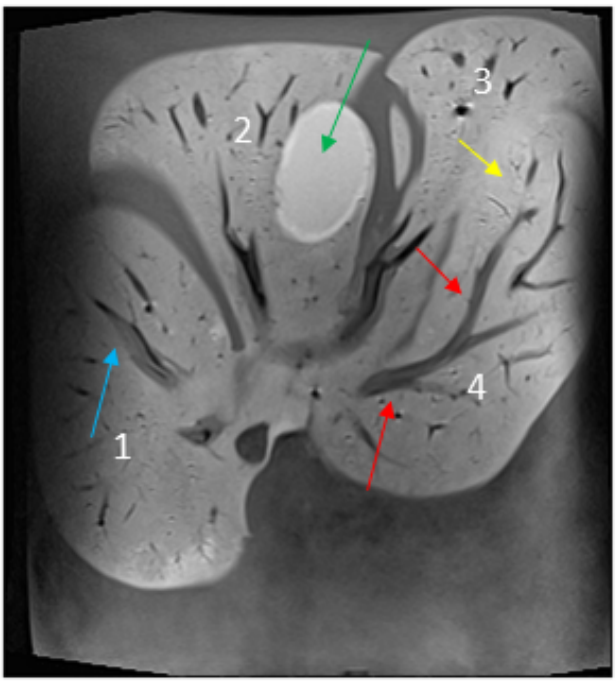


Figure 21: A single slice of the T1-weighted scan of the liver perfused with saline solution. The scan was performed using a 2D spin echo sequence. The four liver lobes are indicated. The red arrows indicate a branch of the hepatic artery, the blue arrow a branch of the portal vein. These branch off into smaller vessels, indicated by the yellow arrow. The gallbladder is annotated by the green arrow.

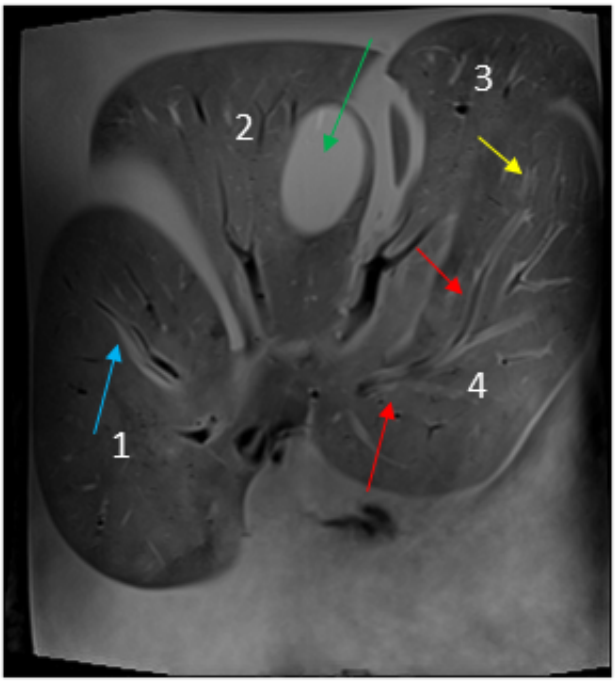


Figure 22: A single slice of the T2-weighted scan of the liver perfused with saline solution. The scan was performed using a 2D turbo spin echo sequence. The four liver lobes are indicated. The red arrows indicate a branch of the hepatic artery, the blue arrow a branch of the portal vein. These branch off into smaller vessels, indicated by the yellow arrow. The gallbladder is annotated by the green arrow.

Angiography Figure 23 shows six images taken with the FLASH sequence taken over time, taken approximately 4.2 seconds apart. Again, the typical porcine liver anatomy with its four lobes is visible, but not as clearly as in the previously presented scans. The contrast agent slowly reached the liver from the control room and these images show enhanced contrast in the liver vasculature. From image B onwards, you can see enhanced contrast in the smaller vascular structures of the liver.

Since the perfusate — in this case, saline solution with the contrast agent — flows out of the inferior vena cava of the liver, some of the contrast agent eventually ends up in the reservoir, surrounding the liver. This reduces the visual contrast between the vasculature and surrounding tissue in image F. Because of that, the contrast agent seems relatively homogeneously dispersed throughout the liver at first. However, some vessels appear brighter than the — for example, the liver region in the top right of image F seems brighter than the bottom right region. The $T1$ scan performed after the administration of gadolinium and the quantification of perfusion using the $T2^*$ scans will show the difference between these regions in more detail.

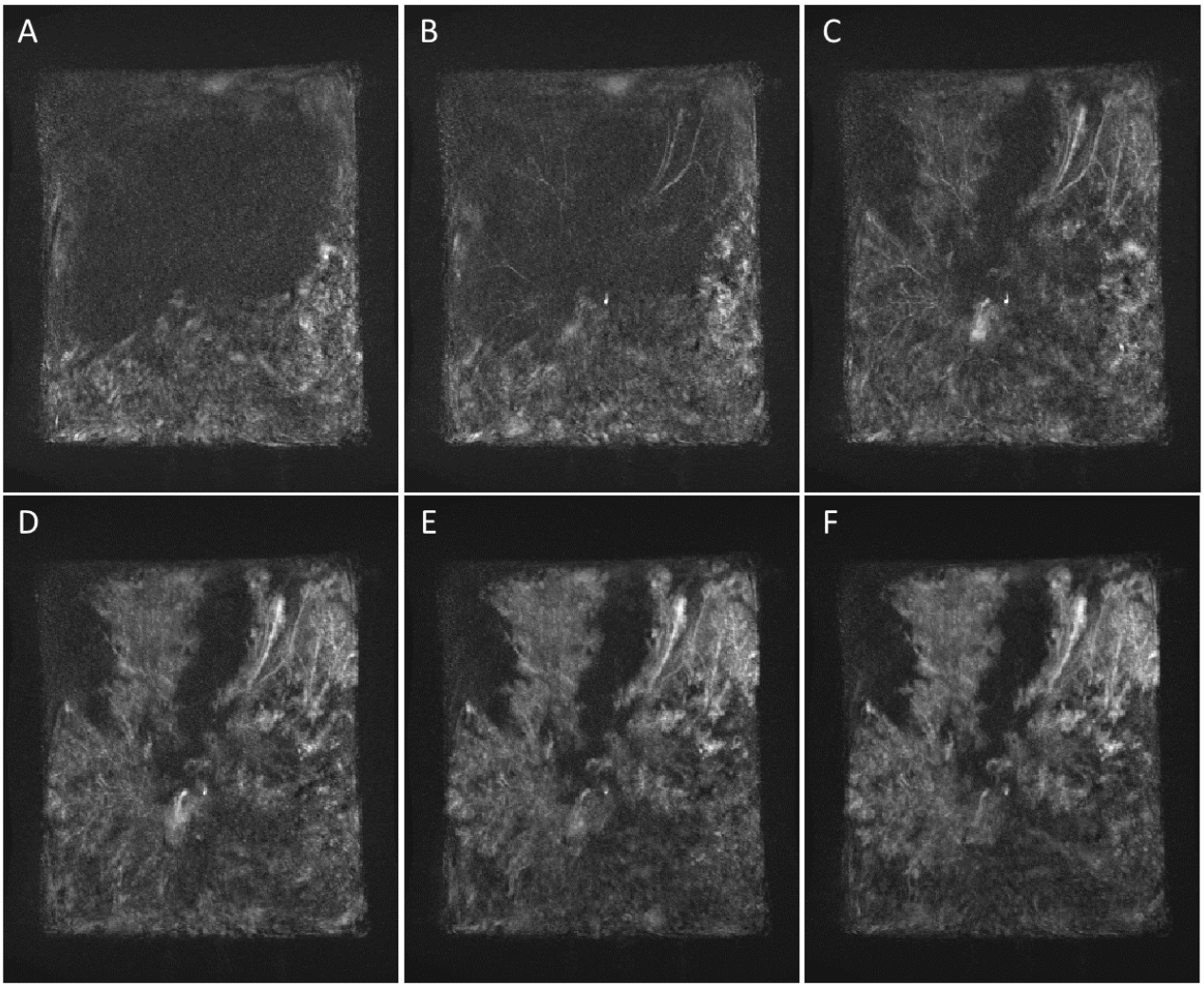


Figure 23: Results of the contrast-enhanced angiography scans of one slice over time of a liver perfused with saline solution. A FLASH-sequence is used and maximum-intensity projections are presented here. The contrast agent Dotarem slowly enters the vasculature of the liver through the arterial cannula. Frames are taken approximately 4.2 seconds apart.

Liver malperfusion Figure 24 shows a $T1$-weighted scan performed after contrast agent administration. Some of the contrast agent accumulated in the liver tissue, which gave increased contrast in soft liver tissue surrounding the vasculature. This scan shows several dark regions (some examples are circled in red) which received little to no contrast agent, while other regions (that show up brighter in this image) received a lot more. This indicates that these regions received little to no perfusate, and that the liver is therefore not homogeneously perfused.

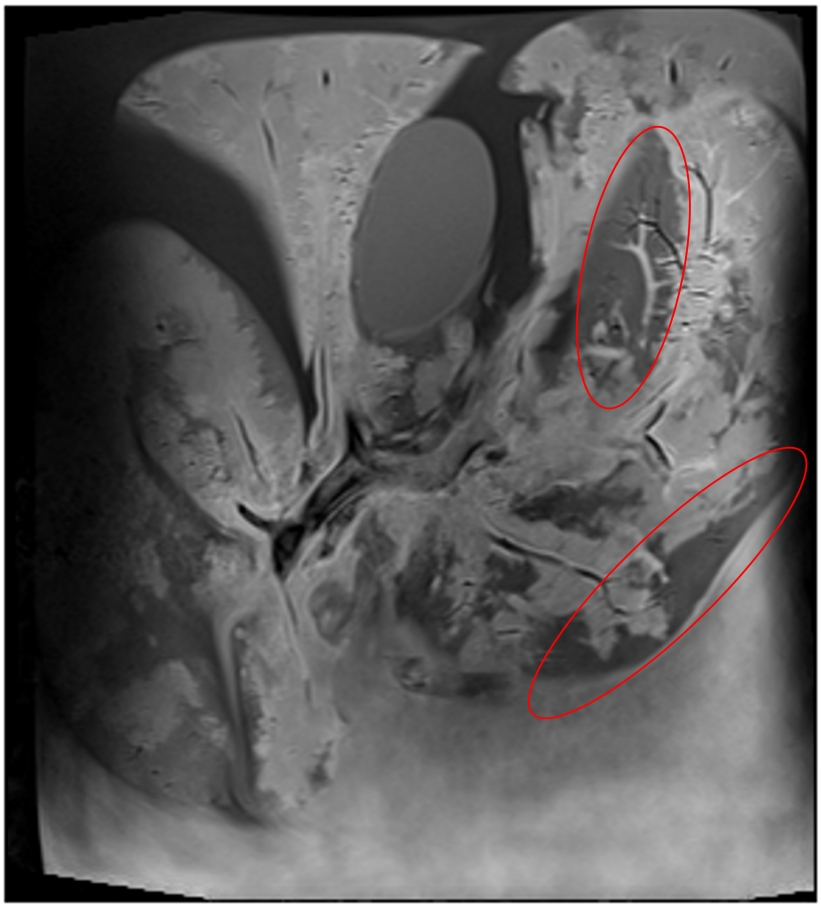


Figure 24: A single slice of the T1-weighted scan of the liver perfused with saline solution after administering the contrast agent Dotarem. The scan was performed using a 2D turbo spin echo sequence. Regions with decreased perfusion (circled in red) showed up dark in this image.

Quantification of perfusion The left image of figure 25 shows the four liver lobes selected as regions of interest in the $T2^*$ scan. The time-intensity curves (see graph on the right in figure 25) show that lobe 2 (green) received the most amount of contrast agent, since it shows the highest peak in tissue perfusion factor over time. The other three lobes received less contrast, showing similar $STPF$ values, all approximately a factor 1.6 lower than lobe 2. These values decrease over time — as contrast agent is washed out — at similar rates.

In figure 24, darker regions indicating malperfusion could be observed. Figure 26 shows the results of perfusion quantification of these two badly perfused regions (red and green) compared to a neighbouring region that showed up brighter on the $T1$ image. The time-intensity curve shows significantly lower peaks in scaled tissue perfusion factor for the malperfused regions than the brighter region — 2.7 times lower for the red region and 4.6 times lower for the green region. This confirms and quantifies the observation that the liver was not homogeneously perfused.

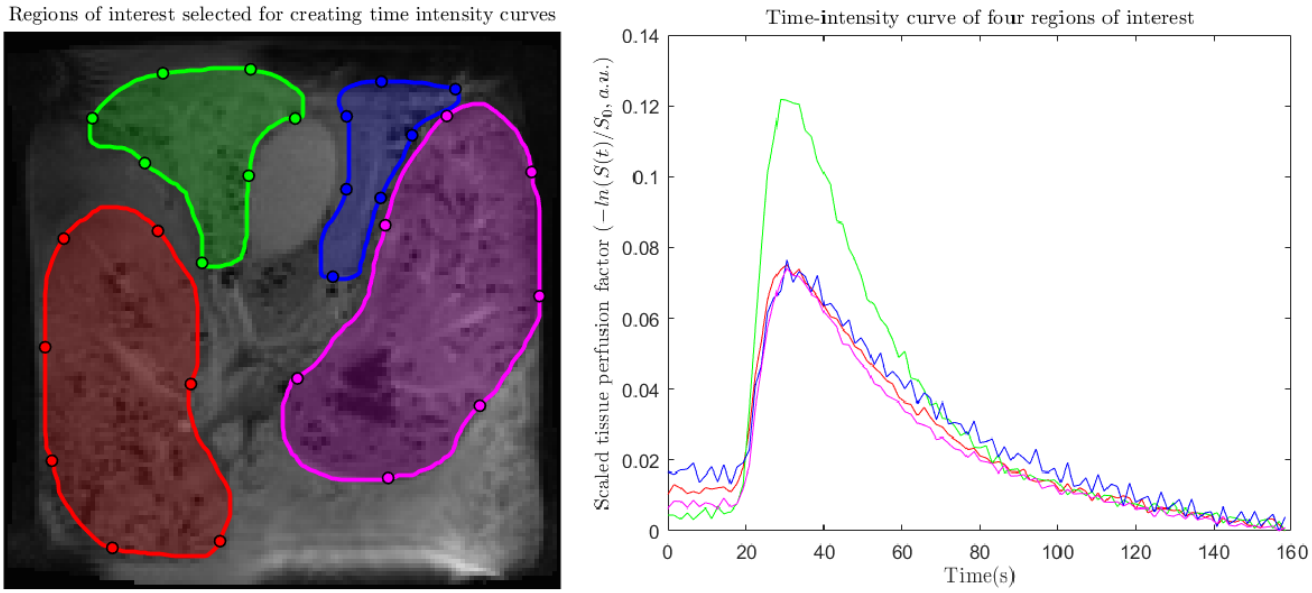


Figure 25: Left: The four liver lobes selected as regions of interest for perfusion quantification by time-intensity curves. Right: Time-intensity curves showing the scaled tissue perfusion factor of each liver lobe over time. The liver lobe indicated in green received a higher dose of contrast agent than the other three lobes, showing a higher peak in STPF.

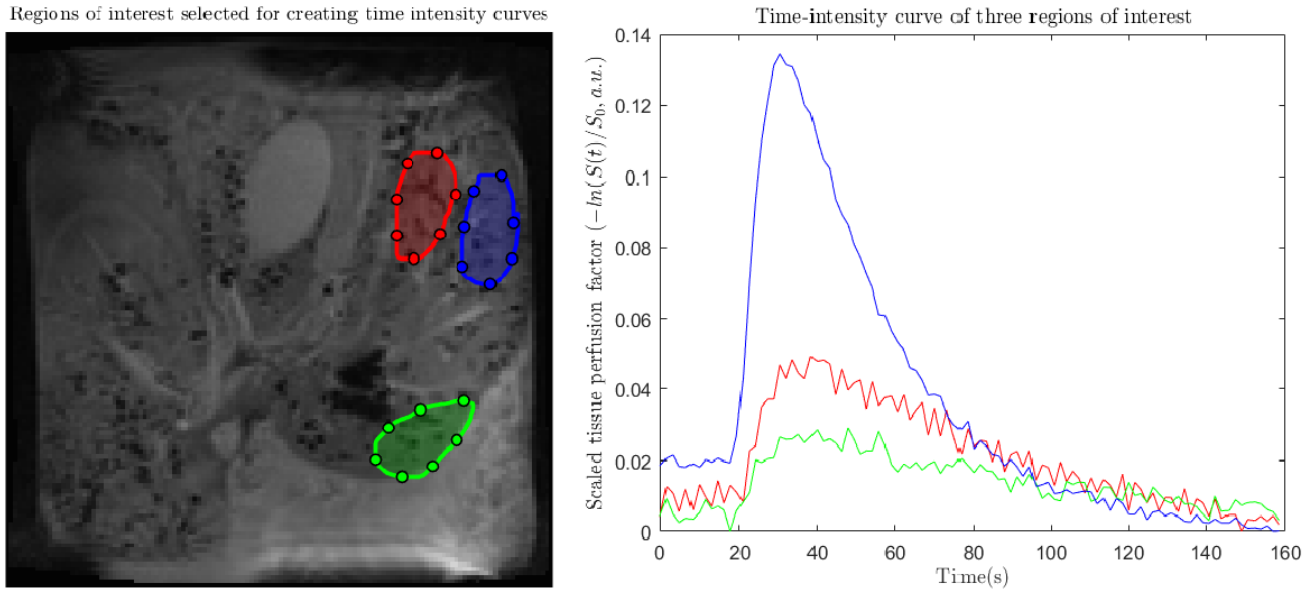


Figure 26: Left: Three regions selected as regions of interest for perfusion quantification by time-intensity curves. Red and green regions correspond wither darker, malperfused regions indicated on the scan post-contrast. The neighbouring blue region showed up lighter on this scan and should be better perfused. Right: Time-intensity curves showing the scaled tissue perfusion factor of each selected region of interest over time. The regions of malperfusion (red and green) showed significantly lower peaks in scaled tissue perfusion factor over time compared to the blue region.

6.3.2 Blood perfusion

Angiography Figure 27 shows several of the angiography scans over time performed using the FLASH-sequence. This time, the contrast agent was administered through the venous line, since there was a substantial amount of blood leaking out of the proper hepatic artery (starting to become visible by the streaks on the left of image D). Through the venous cannula, some blood with contrast agent ended up in the liver vasculature over time. On the top of images D and E, small white spots start to become visible, indicating the presence of more leaks. There were most likely lacerations within the liver originating from rough handling at the slaughterhouse. These became larger during perfusion at high pressure and started leaking blood.

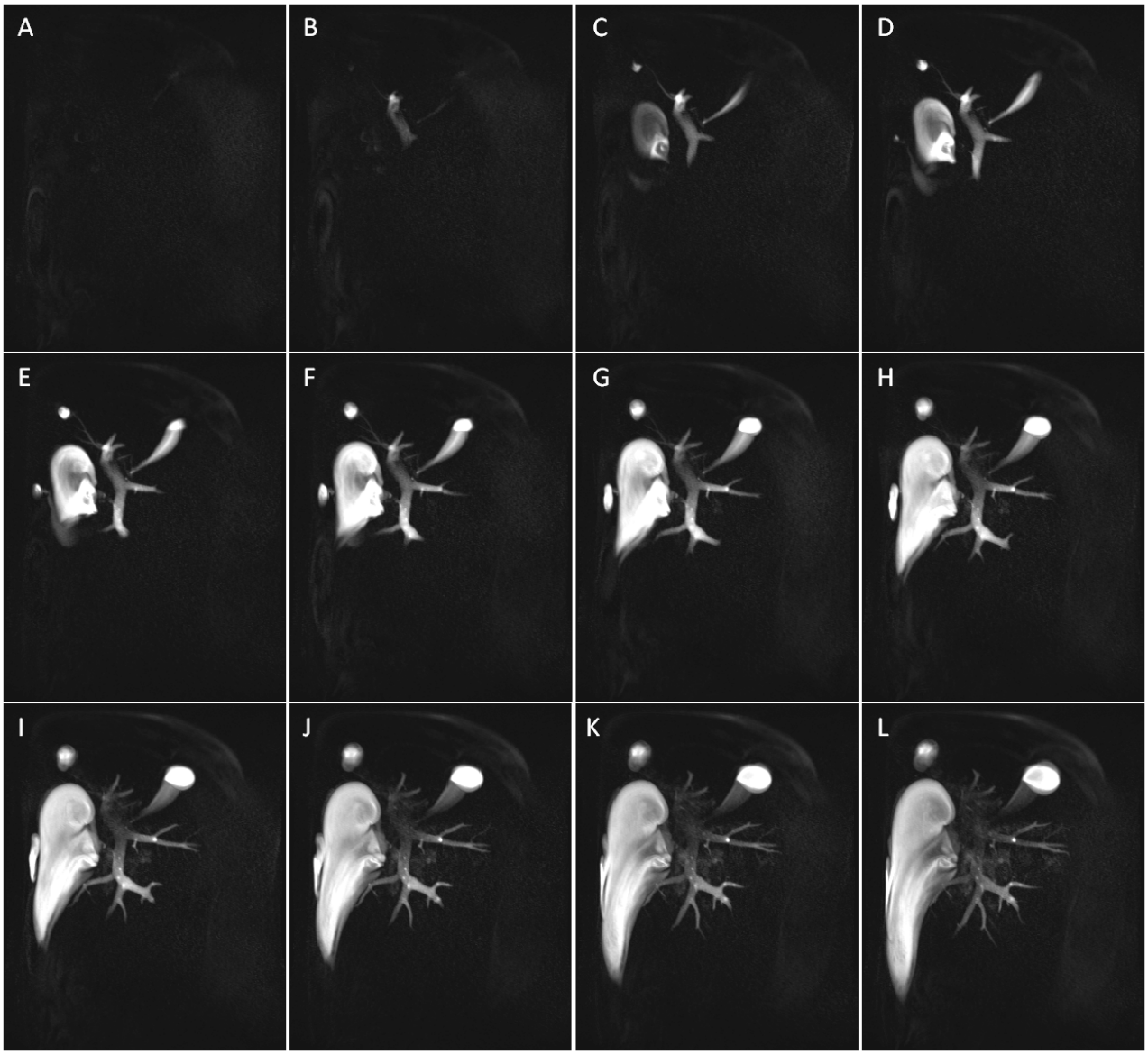


Figure 27: Results of the contrast-enhanced angiography scans of one slice over time of a liver perfused with blood. A FLASH-sequence was used and maximum-intensity projections are presented here. The contrast agent Dotarem slowly enters the vasculature of the liver through the venous cannula. The frames shown here are taken approximately 4.2 seconds apart (some intermediate scans are left out for visualisation). On the left, the leak at the arterial cannula starts to become visible from image D onwards. Two additional smaller leaks start to appear (starting from images D and E) above the main vascular branch, indicating the presence of lesions within the liver.

6.4 Discussion and recommendations

6.4.1 Summary of results

In this experimental phase, the system has been proven suitable for the perfusion of porcine livers during MRI scanning. MRI scans were made using different sequences to visualise liver anatomy and to perform perfusion scans with the use of a gadolinium-based contrast agent.

During perfusion with saline solution, the liver vasculature was visualised by performing angiography scans while injecting contrast into the system from the control room side. A $T1$-weigthed scan performed after injection of the contrast agent indicated that several regions were not perfused as well as others, which was not strange given that this liver was obtained from the slaughterhouse 24 hours before scanning. This was confirmed by quantifying perfusion, by creating plots of the scaled tissue perfusion factor (representing the change signal intensity due to contrast agent) over time of different regions of interest.

The same scans were performed on a liver perfused with blood. On the contrast-enhanced scan, a leak at the height of the proper hepatic artery was visible. Angiography scans performed during the injection of contrast on the venous side showed some contrast still going into the liver and showed lesions within the liver.

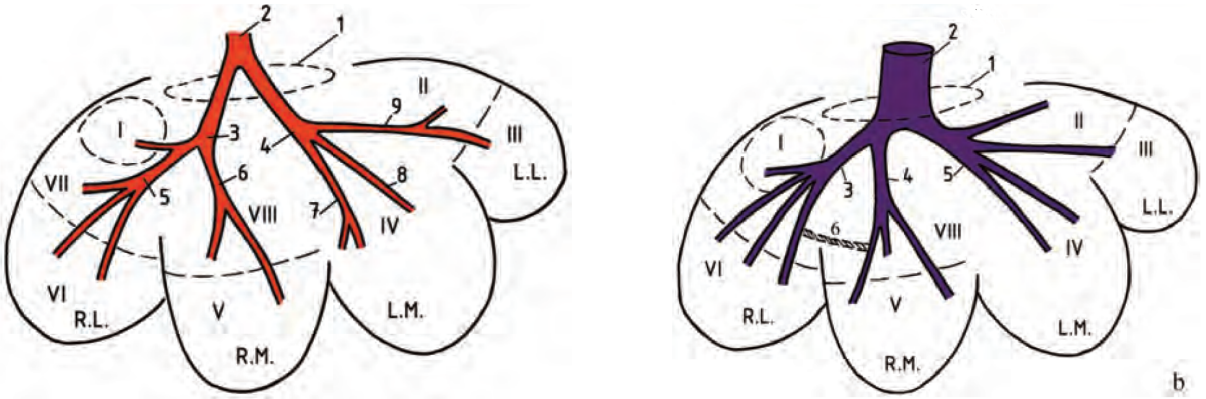
6.4.2 Homogeneity of perfusion

The angiography scans during saline perfusion (figure 23) showed that there was contrast agent arriving into the liver vasculature, indicating functional perfusion. These scans also indicated inhomogeneity of perfusion, showing brighter and darker regions within the liver. However, as the resolution was relatively low (with a pixel spacing of 1.1719 mm in both x and y directions), this conclusion could not be made with great certainty. Investigating the $STPF$ values over time based on the $T2^*$ scans performed during contrast administration confirmed this conclusion; while three of the four lobes were equally perfused, one of the lobes contained a with peak $STPF$ values up to 4.6 times higher than a neighbouring region 26. Since both livers were scanned over 24 hours after retrieval from the slaughterhouse and were not flushed to keep the vessels open for longer, this is not surprising. It is recommended to repeat this scanning process and quantification of perfusion on livers prepared and flushed as described in chapter 4. Once results for a viable and well-perfused liver are obtained, they should be compared to the results of this inhomogeneously perfused liver.

6.4.3 Comparison to literature

Liver anatomy and vasculature Figure 28 shows two sketches of a porcine liver.[55] These sketches indicate the division of the liver into four lobes and eight segments and the branches of the hepatic artery (left) and portal vein (right).

When comparing figure 21 to these sketches, the four liver lobes can be distinguished clearly. Additionally, the way the vessels branch out also looks very similar to the left sketch, especially how the right hepatic artery branches out in lobe 4. When looking at the angiography scan of the same liver, small vessels do not become much more visible due to the limited spatial resolution. However, for the liver perfused with blood, the larger branches of the portal vein can be distinguished on the angiography scan quite clearly (see figure 27).



Abbreviations: 1, hilus; 2, hepatic artery; 3, right hepatic artery; 4, left hepatic artery; 5, ramus lateralis; 6, ramus medialis; 7, ramus quadratus; 8, ramus lobi medialis sinistri; 9, ramus lobi lateralis sinistri; LL, left lateral; LM, left median; RL, right lateral; RM, right median

Abbreviations: **(a):** 1, portal vein; 2, left portal vein; 3, right portal vein, for **(b):**1, hilus; 2, portal vein; 3, ramus (R.) dorsalis dexter; 4, R. ventralis dexter; 5, R. sinister; 6, communicating branches of the portal vein; LL, left lateral; LM, left median; RL, right lateral; RM, right median

Figure 28: Sketches of the hepatic artery (red, left) and portal vein (blue, right) branches in the porcine liver. These two sketches also show the anatomical structure of the liver divided into four lobes, subdivided into eight segments.[55]

Quantification of perfusion Figure 29 shows curves of computed contrast agent concentrations over time, based on both $T1$ and $T2^*$ relaxation rates; $R1$ (squares and crosses) and $R2^*$ (black dots).[56] In this study, the same contrast agent was used (Dotarem), but this study used isolated perfused rat livers. These graphs show that if a high concentration of contrast agent is administered (12mM in this case), the effects on $R1$ and $R2^*$ differ. This indicates that the effect on $T2^*$ relaxation starts to dominate with a higher gadolinium concentration. Since the concentration does not increase over time, the rate with which both relaxation rates increase remains the same, but $R2^*$ relaxation rate is increased more than $R1$.

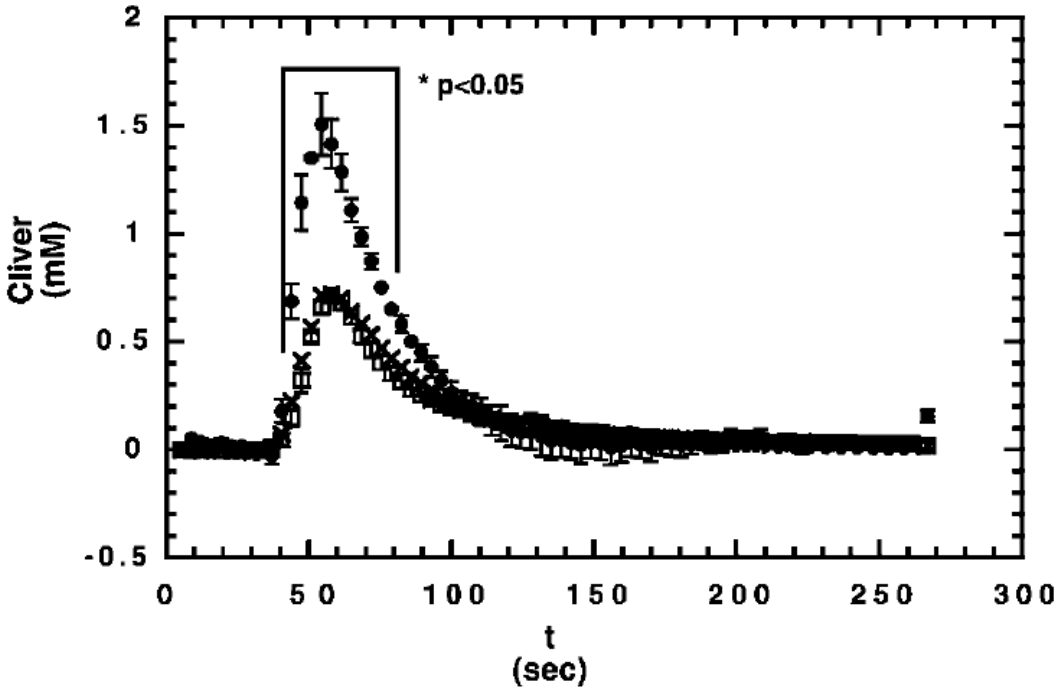


Figure 29: Plots of contrast agent concentration over time obtained from MRI scans of an isolated perfused rat liver. Results are obtained from both $T1$ relaxation rate (squares and crosses) and $T2^*$ relaxation (black dots). 12mM of Dotarem was administered, resulting in different graphs for the $T1$ and $T2^*$-weighted scans.[56]

When comparing the results presented in figure 26 to this reference, a similar shape can be observed, especially for the well-perfused region shown in blue. The $STPF$ values cannot be directly compared to this reference, as they only represent an approximation of the change in signal intensity due to the contrast agent. To obtain exact contrast agent concentrations in this reference, $R1$ and $R2^*$ relaxation times were calculated. $R2^*$ values were calculated from several scans with different echo times and a fixed excitation angle.[56]

Instead of using $T2^*$-weighted scans, plots of contrast agent concentration over time can also be derived from contrast-enhanced $T1$-weighted scans. $R1$ values can be calculated from several scans with excitation angles and a minimal echo time.[56] With a lower contrast agent concentration — at least below 6mM — the results will be very similar to those derived from $T2^*$ scans. It would be a valuable validation to perform both $T1$ and $T2^*$ scans on the isolated perfused pig liver, to apply the same method for calculating contrast agent concentration, and to compare results from both methods.

6.4.4 Field of view

During the perfusion with blood, the liver obtained from the slaughterhouse was quite large. A large reservoir with a volume of 40L was used during this experiment, but the edges of the liver started to curl up on the edges of the reservoir (also visible on figures 21 and 22).

Figures A8 and A9 in appendix A.9 show the $T1$ and $T2$ images made during this experiment and some artefacts around the edges of the reservoir can be observed (indicated by the red arrows). To fit the entire liver on one MRI image, a large field-of-view was chosen with a length of 384 mm. Since the magnet tunnel for this MR scanner is relatively small (700 mm), the magnetic field lines start to bend at the edges of this tunnel (see figure A10 in appendix A.9). As the field-of-view is chosen to be so large, these field lines cause artefacts at the edges of the field-of-view.

There is little control over the size of a pig's liver when obtaining it from a slaughterhouse. However, the design of the liver reservoir can help reduce the field-of-view required for MRI scans. A bowl-shaped reservoir as recommended in the literature review[15,21] or a reservoir with tapered edges would help with reducing the blood volume, making it easier to prevent erythrocyte sedimentation. However, such designs would still make the edges of the liver curl upward along the shape of the reservoir and the top part would need to be big to fit the liver. An alternative liver reservoir design could consist of a bag surrounding the liver instead of a solid reservoir. That way, the liver can lay flat on the MRI bed while still being submerged in perfusate. However, one challenge with this design is connecting the lines of tubing to the reservoir and keeping them in place. It is advised to try out different reservoir designs to see what fits the porcine livers, perfusate volume, and MRI bore size best.

6.4.5 Leaking perfusate

From image 27, several leaks could be observed. There was a substantial leak in the hilus of the liver, near the proper hepatic artery. During visual inspection of the liver before perfusion, there were no leaks at the cannula itself. However, when pressure was applied using a syringe, small leaks became visible at the location where the hepatic artery goes deeper into the liver tissue. These leaks worsened during perfusion, during which an even higher pressure was supplied. There is still a learning curve regarding the liver preparation procedure, especially if perfusion must be started within 5 hours of ischemia time for viability testing.

Two additional leaks were visible on the top of each angiography image 27. This indicated the presence of lacerations within the liver, possibly caused during the dissection of the organ package out of the pig at the slaughterhouse or during transport. They were not visible by eye before perfusion and they most likely worsened during perfusion due to the high blood pressure.

6.4.6 Prospects of magnetic resonance imaging

This chapter has demonstrated various methods of assessing blood flow in isolated perfused livers using MRI — both qualitatively and quantitatively. However, the current sequences did not provide a great spatial resolution. To image the smaller vessels in more detail and track perfusion in these regions over time, 4D flow MRI can be applied. This will enable both the visualisation of vascular structures and the quantification of pulsatile flow patterns within a scanned 3D volume[57] at the same time. To synchronise MRI data acquisition with the pulsatile flow, it is recommended to perform external triggering.[58] The pulsatile waveform generated by the arterial pump is then converted to a signal with which MRI images are obtained.

The final step regarding MRI imaging of livers perfused with this setup would be the injection of microspheres loaded with a radioactive isotope such as holmium, mimicking radioembolisation therapy in an ex-vivo environment. Combining the injection of these microspheres with the quantification of perfusion using $T2^*$ will allow for the study of the distribution of the microspheres throughout the liver. One aspect that must be taken into account here is the homogeneity of perfusion. In non-homogeneously perfuse liver, microspheres do not have the chance to end up in regions that receive no blood, so the resulting holmium distribution would be biased. Radioembolisation experiments must therefore be performed on livers that are homogeneously perfused. This also means that our own definition of a viable liver must apply, since these parameters determine whether all parts of the liver are considered viable and are properly perfused. This underlines the importance of combining MRI experiments with viability testing and the method of preparing and perfusing livers described in chapter 4. Once, according to the blood parameters, livers are considered viable, homogeneity of perfusion should be assessed using one of the methods described on this chapter. When it is clear that all viability-parameters are taken into account, the perfusion system is ready for studying the distribution of microspheres throughout the liver.

6.4.7 Imaging-based organ viability testing

As was concluded from the systematic literature review, liver viability can be assessed using imaging modalities other than MRI as well, for example, ultrasound imaging. Dynamic contrast-enhanced ultrasound (DCEUS) imaging allows for contrast enhancement patterns to be characterized with a higher temporal resolution than MRI.[59] In one of the studies included in the review, similar time-intensity curves have been created using DCEUS with a temporal resolution almost 20 times higher[26] than the curves obtained from MRI presented here (see figure 30). Using DCEUS, changes in arterial and venous flow over time can be visualised that cannot be observed with the naked eye or using MRI. Although the temporal resolution obtained with US is much higher than that obtained with MRI, the spatial resolution is similar, approximately 1 mm.[26]

However, one drawback compared to MRI is the fact that current DCEUS quantification scans ultrasound scans provide two-dimensional spatial data. There is evidence for the feasibility of real-time 3D DCEUS scans in in-vitro flow models, but the quantification of flow in-vivo or in dynamic situation such as isolated perfused organs still requires more research.[60]

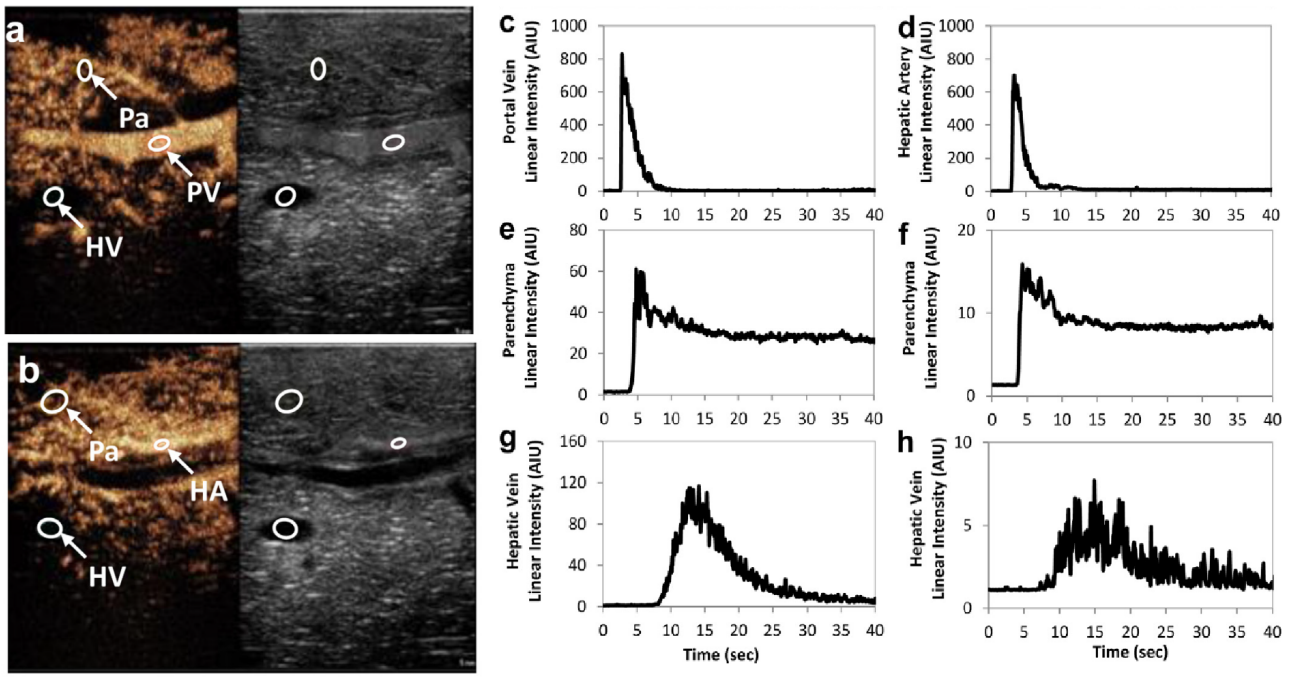


Figure 30: Time-intensity curves showing the progression of contrast microbubbles through the liver, visualised using DCEUS imaging.[26] Regions of interest have been selected (circled in images A and B) in the portal vein (C), hepatic artery (D), liver parenchyhma (E and F) and hepatic vein (G and H).

Ultrasound contrast can be tuned slightly using microbubble size, providing contrast in larger vessels or microvasculature. Choosing the correct microbubble size is important for accurate quantification of perfusion, since microbubbles persisting in liver tissue after perfusate washout can cause disrupted time-intensity curves.[26]

An additional US method described in the systematic review that does not make use of microbubbles is Doppler ultrasound, with which arterial and venous blood flow velocities can be quantified.[22] However, for isolated organ perfusion, this method must be investigated in more detail. According to research, changes in liver parenchyma flow have not yet been observed over long periods of perfusion,[22] and in some studies, the technique has been only applied in a qualitative way.[16,20]

For qualitative conclusions of perfusion with an even higher spatial resolution, computed tomography might be the best option. This modality is able to visualise overall liver perfusion with an excellent spatial resolution of 0.14 – 0.22 mm,[61] depending on the device. Several papers in the systematic review specified the use of CT for image-based viability testing, leading to high-resolution images of vascular structures in the liver, as shown in figure 31.[27] One disadvantage of CT would be the limited ability for quantification since there is little standardisation on the technique used. Many physiological changes in liver tissue can occur from the use of iodinated contrast agents, which should be investigated in more detail.[27]

The choice for the optimal imaging modality for viability testing thus depends on the application. MRI, CT and US are all able to provide qualitative conclusions on perfusion, with CT giving the highest spatial resolution. When accurately quantifying changes in flow patterns over time, ultrasound is more suitable, although this is currently only the case for 2D scans. MRI serves as a good middle ground regarding spatial and temporal resolution, being able to provide 3D perfusion scans. MRI also is flexible regarding the multiple ways flow can be quantified.

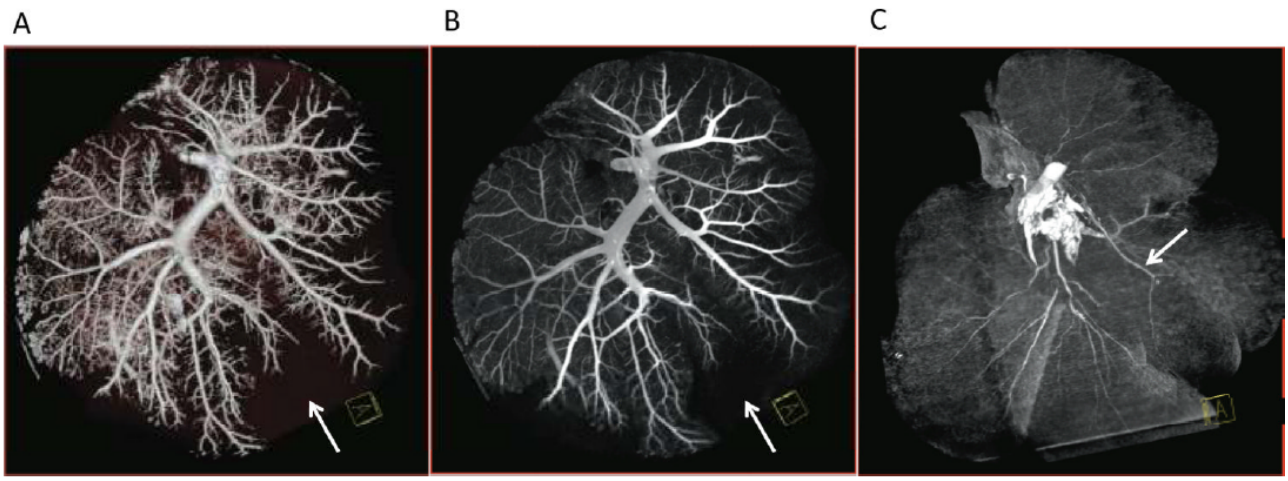


Figure 31: Maxiumum intensity projection images of hepatic vasculature obtained using a contrast-enhanced CT angiogram. Branching of the portal vein (A in 3D and B 2D with contrast enhancement, arrow indicates the gallbladder covering the vasculature) and hepatic artery (C, 2D with contrast enhancement, arrow indicates cystic artery approaching the gallbladder) are shown.[27]

6.5 Conclusions

This chapter has demonstrated that the system is suitable for liver perfusion in the MRI room. During perfusion of porcine livers with saline solution or blood, perfusion was assessed using MRI by applying different imaging sequences. Scans performed after contrast agent administration were used for qualitative assessment of perfusion, concluding that the liver was not homogeneously perfused. This conclusion was confirmed and quantified by plotting a scaled tissue perfusion factor — which approximates contrast agent concentration — over time. This analysis indicated that one lobe overall received more contrast agent than the other three lobes and that within one of the lobes, some regions received up to 4.6 less contrast agent than other regions.

Although some MRI methods need more investigation for proper application for imaging isolated perfused livers, this imaging modality can be used for quantifying perfusion in isolated perfused livers. Especially if $T1$ and $T2^*$ relaxation times can be determined, flow rates can be quantified accurately using imaging.[56] Before this system can be used for investigating the distribution of microspheres loaded with radioactive holmium during radioembolisation, homogeneous perfusion of the livers must be reached.

Besides using MRI, there are other imaging modalities that can be used to assess liver viability. For providing good qualitative conclusions on perfusion — for example looking at perfusion in fine microvasculature structures — computed tomography is able to provide detailed 3D scans with great spatial resolution. When flow rates are to be quantified — for example, to visualise complicated flow pattern changes over time — ultrasound imaging is the most suitable modality due to its high spatial resolution.

7 Discussion and recommendations

7.1 Analysis of research questions

This section will answer the sub-questions that were stated in the introduction to be able to answer the main research question: How should a physiologically relevant ex-vivo perfusion platform be designed and implemented that can keep a liver viable and functional for four to six hours while applying magnetic resonance imaging?

7.1.1 Functional requirements

MRI-related design requirements and design features To create a list of functional requirements for this perfusion platform, inspiration was drawn from existing systems used in medical imaging by conducting a systematic literature review. A list of functional requirements was defined regarding the safe use of the platform in an MRI scanner. For example, no metal-bearing parts could enter the MRI room, which was realised by separating the liver reservoir in the MRI scanner from the driving system in the control room.

As suggested in the review, the driving system can also be placed on 0.5 mT control line. In this experiment, the tubes were fed through holes in the wall dividing the MRI room from the control room. However, since long lines of tubing did provide additional resistance to the blood flow, placing the system on the control line would be a good option to reduce the required tubing length.

Additional design features Another useful reservoir design to implement in the future is a bowl-shaped reservoir that contains a double wall for temperature regulation.[15] An additional safety feature would be a system that shuts down the pumps when the blood/perfusate level in the reservoir gets too low. This prevents pump failure by preventing air from begin pumped into the system. Such a system can be implemented by placing a sensor on the side of the reservoir that keeps track of the blood/perfusate level, coupled with an interface that turns off the pumps.

Arterial filters used in extracorporeal membrane oxygenation can be used as a second safety feature. These are used for filtering out air bubbles and any debris or blood clots present in the blood that might otherwise get stuck in the pump heads. They provide a slight additional resistance to the blood flow (ranging from 3.0 to 5.1 mmHg per mL/min of blood flow, depending on the type).[62] If this cannot be overcome by altering pump settings, it is recommended to filter the blood for several minutes and then removing the filters for the remaining duration of perfusion.

7.1.2 Physiologically relevant environment

Creating a physiologically relevant environment for the isolated perfused liver is complex. Since this environment directly affects viability testing results, the answers to the (sub)-questions related to liver viability also fall back on the principle of what a liver needs to function properly.

To answer these questions, inspiration was drawn from the systematic literature review. The overview presented a multitude of methods for mimicking the blood supply to the liver. These include mimicking the physiological flow and pressure waveforms, maintaining body temperature, and adding oxygen and other additives to the perfusate for proper gas exchange and liver metabolism.

7.1.3 Liver viability

As was stated before, liver viability testing results are heavily dependent on the physiologically relevant environment that is created for the liver. Besides the insight into the in-vivo aspects that must be mimicked to keep livers viable, the methods with which liver viability is assessed are equally important. That is why — for the purpose of this thesis and further work performed on this setup — a clear and concise list of criteria has been set that must be met to regard a liver as viable when perfused by the in-house system. This definition answers both the question regarding the conditions that indicate a viable liver and the methods with which viability is to be tested.

Definition of a viable liver A liver is regarded as viable when the following properties have been reached during perfusion:

- Homogeneous perfusion of blood throughout the liver tissue. This can be ascertained by performing contrast-enhanced imaging of the liver vasculature, either using MRI[21] or US[26] during perfusion. The contrast agent enters the liver through the branches of the hepatic artery and/or portal vein, after which it should reach the microvasculature. If the liver lobes are homogeneously perfused, equal amounts of contrast agent arrive in the center and near the edges of the lobes.
- Maintenance of arterial and venous flow rates and pressure values as follows:
  - Hepatic arterial flow should be pulsatile, with a lower limit of 60 mL/min, an upper limit of 1000 mL/min, and a mean flow of 250 mL/min.
  - Portal venous flow should be around 1200 – 1300 mL/min.
  - Hepatic arterial pressure should be pulsatile, with a lower limit of 70 mmHg, an upper limit of 100 mmHg, and a mean pressure of 85 mmHg.
  - Portal venous pressure should be around 5 – 10 mmHg.
- Maintenance of arterial pressure and flow waveforms, mimicking the systole and diastole of the heart.
- Maintenance of liver temperature. Regulation of perfusate temperature around 37 °C for normothermic perfusion is recommended. When the organ stays around body temperature, the liver metabolism will occur as normal, so blood parameters can serve as accurate indicators of liver viability.
- Maintenance of gas exchange levels, reflected in the following blood parameters:
  - Blood pH should be around 7.35 – 7.45.[14,20]
  - Partial oxygen pressure should be around 75 – 185 mmHg.[14,47]
  - Partial carbon dioxide pressure should be around 36 – 45 mmHg.[14,47]
  - Bicarbonate levels should be around 16 – 28 mmol/L.[48]
  - Base excess levels should be around -2.5 – +2.5 mmol/L.[47]
  - Oxygen levels should be completely (100%) saturated.[49]
  - Total carbon dioxide concentration should approach 10 – 40 mmol/L.[51]
- Maintenance of homeostasis, reflected in blood electrolyte and glucose levels.
  - Sodium levels should approach 138 – 158 mmol/L.[49,50]
  - Potassium levels should approach 2.3 – 6.7 mmol/L.[47,49,50]
  - Calcium levels should approach 1.13 – 1.3 mmol/L.[47]
  - Glucose levels should approach 4 – 15 mmol/L.[14,47]
- Maintenance of blood hemoglobin levels between 27.5 – 31.9 %PCV.[50]
- Maintenance of blood hematocrit levels between 5.0 – 9.0 g/dL.[49,50]
- Maintenance of lactate clearance. Blood lactate levels should decrease to concentrations below 1.7 mmol/L.[16]
- Maintenance of bile production with a continuous secretion rate of 4.2 $\pm$ 3.4 mL/h.[24–26]
- Absence of oedema. This can be visualised on anatomical MRI scans[15,21] or assessed by weighing the liver during perfusion.

Analysis of viability and viability testing Several elements of this definition have been assessed in this thesis, such as blood parameters — using the i-STAT alinity — and homogeneity of perfusion — using contrast enhanced MRI. Several elements are still to be tested on livers perfused by this setup in future experiments. For example, oedema in the liver has not been assessed in this research. The easiest method to keep track of water retention would be to weigh the liver during perfusion, although extreme cases of oedema can be observed under anatomical MRI scans.[15,21]

All blood parameters listed here have been assessed during perfusion, except for lactate clearance. As mentioned in chapter 6, CG4+ cartridges by Abbott are able to measure lactate levels. They are recommended to be used besides the CG8+ cartridges for measuring electrolyte, glucose, hematocrit, and hemoglobin levels.

One liver viability parameter that has not been investigated in this experiment is bile production. When a small cannula is placed in the common bile duct, bile can be collected in a reservoir and the secretion rate can be analysed. Bile production should be secreted with a relatively continuous rate of 4.2 ± 3.4 mL/h.[26] Since this level is reported to stabilise within the first hour of perfusion, it is recommended to measure bile secretion every 15 – 30 minutes to accurately track any changes.

Normothermic perfusion mimics the in-vivo situation most accurately and allows for the most accurate viability testing. It is recommended to use a device such as the Hico Variotherm 550[40] as a thermostat for keeping the perfusate at body temperature (37 °C). This device warms up the water in its basin to the set temperature and — using separate loops of tubing connected to the oxygenators— this device will pump water through the oxygenators and back to its basin. The warm water will surround the fibers in the oxygenator and heat will be transferred to the blood. Even though the water temperature is regulated continuously by this device, it is recommended to measure the temperature of the perfusate and liver as well during perfusion. Water with a higher temperature than 37 °C will be required to keep the liver at body temperature, as loss of thermal energy will occur over the long lines of tubing.

7.1.4 Parameters influencing flow and pressure

An additional aspect of the physiological environment for the liver is the flow rate and pressure of the applied blood flow. As expected, there were several parameters that influenced these hemodynamic parameters during the experiments. For example, the fibers inside the oxygenators have small diameters and they have a low level of compliance due to their rigidity, causing resistance to the blood flow. Combined with additional blood flow resistance of the tubing and the relatively high blood viscosity, this resulted in a low blood flow rate and high blood pressure reaching the liver. Several methods have been introduced for improving these parameters, of which experimentation with pump settings is most important.

7.1.5 MRI experiments

The qualitative and quantitative contrast-enhanced MRI scans described in chapter 6 answer the question of how MRI can be applied to image flow through the liver. Additionally, several recommendations were presented to optimise the imaging of complex flow patterns in the liver using MRI or other imaging modalities. As described in the definition of liver viability, these MRI experiments contribute to the conclusions on liver function by investigating the perfusion in macro-and microvasculature, providing an insight into how homogeneously a liver is perfused. This answers the question of how MRI experiments help provide conclusions on liver function.

When the assessment of perfusion using imaging has been optimised, a more detailed definition of homogeneous perfusion should be defined. This can be done by setting a limit on how much the $STPF$ values or contrast agent concentrations in several regions of interest can differ from each other for a liver to be regarded as homogeneously perfused. It is therefore advised to compare quantitative and qualitative perfusion scans from different livers to get a more detailed indication of what this limit should be. Additional blood parameter measurements can also help set this limit.

7.1.6 Change of liver viability over time

The final question left unanswered is how the viability parameters change over time. The main goal included liver perfusion for four to six hours, which has not been reached in this thesis due to sub-optimal conditions regarding hemodynamic parameters and oxygenation. The following recommendations hold for future experiments, aiming to get more accurate insights into the changes in liver viability during perfusion.

In this thesis, the blood parameters have been investigated every half hour, with an exception of the first hour of perfusion. As most parameters showed great changes within the first hour and stabilised after that (see table 5), it is advised to measure these parameters every 15 minutes instead.

Additional measurements of hemodynamic parameters were performed at the end of perfusion, showing only small variations from the data presented in chapter 4 (measured at the beginning of perfusion). Since both pumps are able to provide stable flows when high pump speeds can be maintained — and short-term changes over time were likely the cause of sensor calibration — these parameters are not likely to change greatly over the time of perfusion in future experiments. However, since the liver was only perfused for 2.5 hours, it is recommended to repeat these measurements when the system is operated for longer periods of time.

It is advised to perform multiple MRI perfusion scans over the course of perfusion. This will help to understand how perfusion in the microvasculature changes over time and if any changes to the system need to be made to improve homogeneous perfusion.

7.2 Liver viability testing during imaging

Added value In this thesis, blood parameters and perfusion scans were performed during separate liver perfusions, under different conditions. It is highly recommended to combine liver viability testing by blood parameters and by applying medical imaging. That way, information can be obtained on both blood parameters and homogeneity of perfusion at the same time. When blood parameters indicate that a liver is viable, but it has not been tested whether there is proper microcirculation of perfusate in the same liver, some regions might be less viable than others. When a liver looks homogeneously perfused on an MRI scan, this does not give a definitive conclusion about metabolic processes in the liver without blood parameter results. Performing blood parameter testing, measurements of hemodynamic parameters, and MRI experiments on the same perfused liver will ensure that all of the elements in the definition of a viable liver are assessed.

System optmisations To obtain reliable conclusions from such an experiment, the in-vivo situation must be mimicked as closely as possible. To achieve this, the following main changes to the perfusate and the system itself must be made:

- Optimising the liver reservoir design to fit large porcine livers while reducing the required field of view for MRI scans.
- Preventing the liver from leaking blood (explained in more detail in the next section).
- Proper oxygenation by applying a lower gas flow rate.
- Adjusting pump settings such as pump speed, pulsatility amplitude, and systole to reach higher flow rates and mimic the pulsatile waveform more closely.
- Using new oxygenators and possibly a larger diameter of tubing (inner diameter of 3/8") to reduce the pressure buildup over the system.
- Regulating temperature using a thermostat such as the Hico Variotherm 550[40] to perform normothermic perfusion.

Radioembolisation The system can also be used for studying microsphere distribution in radioembolisation experiments when these changes have been made. As was discussed before, homogeneous perfusion throughout the liver is required for these experiments. Radioactive holmium in the microspheres is reported to also cause enhanced MR signal decay, increasing $R2^*$ relaxation,[63] so the same methods can be applied to quantify the absorbed radiation dose in different regions of interest.

7.3 Liver preparation

Angiography scans performed on a liver perfused with blood showed significant leakage near the proper hepatic artery (see figure 27). Before perfusion, some leaks deeper within the hilus could be observed when inspecting the liver by eye, while pressure was applied using a syringe filled with water. The decision was made to perform MRI scans anyways. These leaks — and additional lacerations that most likely occurred at the slaughterhouse — worsened when more pressure was applied during perfusion. To prevent such large leaks in future experiments, the following rule of thumb should be taken into account when checking the liver before perfusion: if water only drips out of small vessels very slowly, the liver is still suitable for perfusion and MRI scans. If water spouts out of some vessels when pressure is applied using a syringe, these vessels should be closed off to prevent major leaks. If this is no longer possible, the liver is regarded unfit for perfusion and imaging, and another liver should be prepared for the experiments.

Regarding preparation steps, it might be beneficial to use University of Wisconsin solution for rinsing, as it has been proven to keep livers viable for longer with a smaller chance of oedema occurring.[18,19] It is recommended to perform the initial flushing with Ringer's lactate for example to get rid of blood in the microvasculature, as UW is quite viscous.[32] Due to its high levels of potassium, extra attention should also be paid to electrolyte levels when performing blood tests for viability assessment.[18] However, this solution can also be used well for static cold storage in between flushing at the slaughterhouse and perfusion at the UT.

8 Conclusions

This thesis describes the design, development, and implementation of an in-house system that aimed to keep porcine livers viable while performing MRI scans. To keep a liver viable for several hours of experimentation, the physiologically relevant environment for the liver has been mimicked as closely as possible. With this, a list of all parameters that must be assessed to regard a liver as viable has been presented. Several of these parameters have been investigated in individual liver perfusion experiments. In these experiments, the livers were perfused under sub-optimal conditions for up to 2.5 hours. However, the system design allowed for operation of the setup during MRI experiments.

The work performed on this system has contributed to the understanding of both isolated organ perfusion systems and their implementation into medical imaging. By combining viability testing and MRI scans into one experiment in the future, clearer insights into the influence of certain perfusion parameters on liver viability can be gained. This thesis has described the required optimisations, regarding both the use of the system itself and liver preparation methods. The optimised system in the future will provide a suitable platform for research on the distribution of microspheres in radioembolisation therapy.

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Appendix

A.1 Search logbook systematic literature review

A.1.1 Review goal

To create an overview of design features of existing isolated organ perfusion systems that are used in combination with medical imaging modalities.

A.1.2 Keywords

1. Ex-vivo
2. Animal
3. Organ
4. Perfusion
5. Imaging
6. Normothermic

A.1.3 Synonyms

The table below shows the synonyms of the keywords described above. These include similar and related terms; both broader and narrower terms.

Keyword	1. Ex-vivo	2. Animal	3. Organ	4. Perfusion	5. Imaging	6. Normothermic
	Ex-situ	Porcine	Liver	Perfused	Medical imaging	Physiologic
	Isolated	Bovine	Kidney	Machine perfusion	MRI	Physiological
		Pig	Heart	NMP	Magnetic resonance imaging	Experimental
		Cow		Normothermic machine perfusion	CT	
		Human			Computed tomography	
					US	
					Ultrasound imaging	
					Photoacoustic imaging	
					PA	
					Diagnostic imaging	

A.1.4 Search logbook

The table below is a logbook of all the searches for the systematic literature review. Different searches in the databases Scopus, PubMed and Web of Science have been executed with combinations of different keywords. The used queries were adjusted accordingly, eventually leading to the final query.

Search logbook

Query	Source	# Results	Searched in	Amount	Relevant	Adjustments
TITLE-ABS-KEY ((animal OR porcine OR bovine OR human OR pig OR cow) AND (organ OR liver OR kidney OR heart) AND ("ex vivo perfus*" OR "ex situ perfus*" OR "machine perfus*" OR nmp) AND (imag* OR "medical imag*"))	Scopus	106	Title-abs-key	Not enough	Relevant, too specific	Remove *animal* to see if it gives a lot of difference or not as it feels redundant
TITLE-ABS-KEY ((organ OR liver OR kidney OR heart) AND ("ex vivo perfus*" OR "ex situ perfus*" OR "machine perfus*" OR nmp) AND (imag* OR "medical imag*"))	Scopus	114	Title-abs-key	A lot	Relevant, too specific	Leave *animal* out for now. *"ex vivo perfusion"* as one term might not work, re-phrase and split into two separate keywords
TITLE-ABS-KEY (("ex vivo" OR "ex situ") AND (organ OR liver OR heart OR kidney) AND (perfus* OR nmp OR "machine perfus*") AND (imag* OR "medical imag*"))	Scopus	573	Title-abs-key	Better	More defined regarding ex-vivo perfusion. Very broad approach of imaging	Re-phrase *imag* OR "medical imag*" OR experiment* to include specific imaging modalities
TITLE-ABS-KEY (("ex vivo" OR "ex situ") AND (organ OR liver OR heart OR kidney) AND (perfus* OR nmp OR "machine perfus*") AND (MRI OR "magnetic resonance imaging" OR PA OR "photoacoustic imaging" OR CT OR "computed tomography" OR US OR ultrasound OR "medical imaging"))	Scopus	488	Title-abs-key	Better	Relevant, but imaging still needs to be investigated	Repeat latest query in PubMed to investigate automatic MeSH terms the algorithm comes up with (mainly regarding imaging)
("ex vivo" OR "ex situ") AND (organ OR liver OR heart OR kidney) AND (perfus* OR nmp OR "machine perfus*") AND (MRI OR "magnetic resonance imaging" OR PA OR "photoacoustic imaging" OR CT OR "computed tomography" OR US OR ultrasound OR "medical imaging")	PubMed	1290	All fields	Too much	Some papers irrelevant, check if any abbreviations of imaging modalities have other meanings	Upon assessment of MeSH terms, remove abbreviations of some imaging modalities and add some broad synonyms of *imaging*
("ex vivo" OR "ex situ") AND (organ OR kidney OR liver OR heart) AND (perfus* OR "machine perfus*" OR nmp) AND (imag* OR mri OR "magnetic resonance imaging" OR photoacoustic* OR "computed tomography" OR ultrasound OR "medical imaging" OR "diagnostic imaging")	PubMed	504	All fields	Good amount	Much more relevant	Go back to scopus and apply the following restrictions: - Only journal articles - Year: 2000-2022
TITLE-ABS-KEY (("ex vivo" OR "ex situ") AND (organ OR kidney OR liver OR heart) AND (perfus* OR "machine perfus*" OR nmp) AND (imag* OR mri OR "magnetic resonance imaging" OR photoacoustic* OR "computed tomography" OR ultrasound OR "medical imaging" OR "diagnostic imaging")) Limited to: article only, publication year 2000-2022	Scopus	511	Title-abs-key	Good amount	Relevant, but the way 'ex-vivo perfusion' is phrased now might be too broad	Add the synonym *isolated* to the first keyword (also change the order so the phrasing *"isolated organ perfusion"* becomes clear)

Query	Source	# Results	Searched in	Amount	Relevant	Adjustments
TITLE-ABS-KEY ((isolated OR "ex vivo" OR "ex situ") AND (organ OR kidney OR liver OR heart) AND (perfus* OR "machine perfus*" OR nmp) AND (imag* OR mri OR "magnetic resonance imaging" OR photoacoustic* OR "computed tomography" OR ultrasound OR "medical imaging" OR "diagnostic imaging") Limited to: article only, publication year 2000-2022	Scopus	1404	Title-abs-key	Too much	Relevant, but many articles included on hypothermic perfusion	Now add the term *normotherm* OR physiolog* OR experiment** to specify the papers to physiologically relevant, normothermic perfusion systems or systems for experimental use (instead of clinical)
TITLE-ABS-KEY ((isolated OR "ex vivo" OR "ex situ") AND (organ OR kidney OR liver OR heart) AND (perfus* OR "machine perfus*" OR nmp) AND (imag* OR mri OR "magnetic resonance imaging" OR photoacoustic* OR "computed tomography" OR ultrasound OR "medical imaging" OR "diagnostic imaging") AND (normotherm* OR physiolog* OR experiment*)) Limited to: article only, publication year 2000-2022	Scopus	880	Title-abs-key	Not enough	Now no more review papers or papers published before 2000. Results are relevant.	Terms in this keyword restrict a lot. Remove *physiolog* OR experiment** to see the effect of *normotherm** by itself
TITLE-ABS-KEY ((isolated OR "ex vivo" OR "ex situ") AND (organ OR kidney OR liver OR heart) AND (perfus* OR "machine perfus*" OR nmp) AND (imag* OR mri OR "magnetic resonance imaging" OR photoacoustic* OR "computed tomography" OR ultrasound OR "medical imaging" OR "diagnostic imaging") AND normotherm*) Limited to: article only, publication year 2000-2022	Scopus	32	Title-abs-key	Way too little	Relevant, but too specific. Remove entire keyword to broaden results. Also many papers included on perfusion imaging, try an exclusion	The term *normotherm** filters out a lot! Remove the whole keyword just in case so that no relevant papers are excluded. Also exclude *"perfusion imag*"* as one term to see if this specific branch of imaging can be removed, as this is not relevant to perfusion systems.
TITLE-ABS-KEY ((isolated OR "ex vivo" OR "ex situ") AND (organ OR kidney OR liver OR heart) AND (perfus* OR "machine perfus*" OR nmp) AND (imag* OR mri OR "magnetic resonance imaging" OR photoacoustic* OR "computed tomography" OR ultrasound OR "medical imaging" OR "diagnostic imaging") AND NOT ("perfusion imag*")) Limited to: article only, publication year 2000-2022	Scopus	1217	Title-abs-key	A lot	Relevant, but other relevant papers might be left out	The term *AND NOT "perfusion imag*"* might remove relevant papers, so we remove this term again. Remove the broad term *imag** to remove any studies not related to the specific imaging modalities.
TITLE-ABS-KEY (isolated OR "ex vivo" OR "ex situ") AND (organ OR kidney OR liver OR heart) AND (perfus* OR "machine perfus*" OR nmp) AND (mri OR "magnetic resonance imaging" OR photoacoustic* OR "computed tomography" OR ultrasound OR "medical imaging" OR "diagnostic imaging") Limited to: article only, publication year 2000-2022	Scopus	817	Title-abs-key	Good amount	Relevant	Final query. Irrelevant papers about *perfusion imaging* will be filtered out during paper evaluation. Repeat query in PubMed
(isolated OR "ex vivo" OR "ex situ") AND (organ OR kidney OR liver OR heart) AND (perfus* OR "machine perfus*" OR nmp) AND (mri OR "magnetic resonance imaging" OR photoacoustic* OR "computed tomography" OR ultrasound OR "medical imaging" OR "diagnostic imaging") Limited to: publication year 2000-2022, excluded: review and systematic review	PubMed	573	All fields	Good amount	Relevant, check overlap with Scopus results	Final query. *Journal article* was not an option to refine in PubMed, so instead reviews and systematic reviews were excluded. Repeat in Web of Science
(isolated OR "ex vivo" OR "ex situ") AND (organ OR kidney OR liver OR heart) AND (perfus* OR "machine perfus*" OR nmp) AND (mri OR "magnetic resonance imaging" OR photoacoustic* OR "computed tomography" OR ultrasound OR "medical imaging" OR "diagnostic imaging") Limited to: article only, publication year 2000-2022	Web of Science	996	All fields	Good amount	Relevant, check overlap with Scopus and PubMed results	Final query.

A.2 Results of systematic literature review

	Perfusate		Storage and preparation		Perfusion characteristics	
Authors	Blood characteristics (heparin, anti-coagulant)	Others	Preparation steps	Ischemia time	Normothermic or hypothermic	Perfusion time
Alzaraa et al.	Autologous blood with 5000 units of heparin with additives added during perfusion: parental nutrition, vasodilating prostacyclins, sodium bicarbonate, sodium taurocholate and insulin	No other perfusate used	The system was primed using the autologous heparinized blood. After retrieval, the livers were cold perfused using cold Soltran solution (Baxter Healthcare, Thetford, UK; 1L in PV and 1L in HA) and transported L in PV and on ice. Before connected to the circuit, trapped air and remaining Soltran solution were flushed out using 1L of 0,9% saline solution (Baxter Healthcare, Thetford, UK)	Warm ischemia 19±2 min, cold ischemia 162±24 min (mean values)	Normothermic	6 hours
Averkiou et al.	No blood used	Not specified, most likely the same perfusate as used by Izamis et al.	Static cold storage was less than 1 hour, no further details presented	Under 1 hour cold ischaemia (static cold storage)	Sub-normothermic (room temperature, temperature not regulated)	3 hours
Bu et al.	No blood used	Isotonic crystalloid and colloidal solution, 2000 IU heparin (4L in total)	The livers were immersed in 2L heparinized perfusate and stored in a non-electric cool box at 4 °C for 2-4 hours. After that followed canulation and perfusion.	Less than 30 minutes warm ischaemia, followed by 2-4 hours of cold ischaemia in heparinized perfusate	Normothermic	3 hours
Civale et al.	Blood, Heparin added every 4h, total parental nutrition, prostacyclin, taurocholic acid	No other perfusate used	Not specified, but they most likely followed the protocol described by Butler et al.: After anaesthesia, 15,000 U of heparin was administered and after that blood was collected. Heparin (20,000 U) was administered during the cannulation process. 2L Eurocollins solution (Baxter, Soltran) administered through the PV cannula was used to rinse the livers before complete dissection. While finalising the complete cannulation process, the liver was placed in a bowl at ice temperature	Not specified	Normothermic	Perfusion could be sustained for at least 24 hours, the presented data was obtained in the first 12 hours.
Gaffke et al.	Systemicallly heparinized blood (15000 IU, Liquemin N, Roche, Germany), again anticoagulated outside the body (15000 IU, Liquemin N)	No other perfusate used	After explantation, the livers were preserved in Euro Collins solution (4 °C) on ice until the perfusion set-up. After a short ischemia time, they were then stored refrigerated (4 °C) within a time window of 4 hours.	Not specified in this paper, but based on previous research (Nagel et al.), a warm ischemia time of under 7 minutes followed by a cold ischemia time of 1 hour is expected.	Normothermic	4 hours
Hildebrand et al.	Heparinized blood solution; 700 ml of HTK solution mixed with 300 ml of porcine blood and 20,000 units of heparin.	No other perfusate used	Livers were rinsed with cooled heparinized solution (20,000 units in 2L of HTK solution). After rinsing, livers were transported in a cooled box.	Not specified	Sub-normothermic (room temperature, temperature not regulated)	Not specified
Izamis et al.	No blood used	6L in total: Powdered Williams Medium E (10.8 g/L W4125, Sigma–Aldrich, St. Louis, MO, USA) wo which as been added 1000 IU/L heparin, 2 U/L insulin (Actrapid Penfill, Novo Nordisk, Bagsvaerd, Denmark) and 0.4 mg/L dexamethasone (Dexamed, Medochemie, Limassol, Cyprus) and 2.2 g/L sodium bicarbonate (S5761, Sigma–Aldrich, Medisell, Cyprus) or more within the first hour to reach a pH of 7.3-7.4	Before cannulating, the livers were submerged in an iced slush solution of lactated Ringer's solution (Multi-Pharm, Nicosia, Cyprus). Inverting the liver enabled removal of any air trapped in the hepatic veins. After cannulating, Six to seven liters of ice-cold lactated Ringer's flush solution spiked with 1000 IU/L heparin. After flushing, the livers were disconnected from the tubing and transported in sealed containers.	30 minutes of warm ischemia before 2 hours of static cold storage.	Sub-normothermic (room temperature, no temperature regulation performed)	3 hours
Keravnou et al.	No blood used	See Izamis et al. Same perfusate used here.	After slaugther, livers were flushed with 8L of lactate Ringers solution (6L room temperature, 2L ice cold) through PV and HA. After that, the organs were stored on ice and transported to the lab.	Brief warm ischemia between slaughter and flush with Ringers solution (no time specified). Less than 1 hour of static cold storage during perfusion.	Sub-normothermic (room temperature, no temperature regulation performed), as described by Izamis et al.	4 hours
Liu et al.	Sanguineous perfusion: autologous pig blood diluted with AQIX solution (Imperial College BioIncubator, London, UK) at a hematocrit 9% (hypothermic).	Acellular perfusion: kidney perfusion solution (KPS-1, Organ Recovery Systems, Chicago, Ill, USA). After perfusion with this medium (hypothermic), the livers are flushed with Ringer's solution and then perfused with oxygenated Krebs-Henseleit Bicarbonate (KHB) solution (normothermic)	Sanguineous perfusion: After warm ischemia in-situ, the liver directly went on to be perfused with the blood-solution. No further preparation or rinsing steps described. Acellular perfusion: After warm ischemia ex-situ, livers were flushed with histidine tryptophan ketoglutarate solution (4°C) were procured and placed in the MRI-compatible cassette of the perfusion system.	Sanguineous perfusion: 2 hours of warm ischemia of the left lobe of the liver in-situ (left HA and left PV are clamped). Acellular perfusion: 2 hours of warm ischemia of whole liver ex-situ.	Hypothermic and normothermic (see 'Perfusion time' for sequence)	Sanguineous perfusion: 1 hour of hypothermic perfusion, then 3 hours of normothermic oxygenated perfusion, both with the autologous blood solution. Acellular perfusion: 24 hours with Kidney Perfusion solution (hypothermic), then flushed with Ringers solution, then 1 hour of perfusion with Krebs-Henseleit Bicarbonate solution (normothermic)
Lorton et al.	No blood used	Not directly specified, but VIASPAN kidney perfusate used by Buchs et al.	Organs were preserved in an ice bath, no further details given.	Not specified.	Sub-normothermic (room temperature, no temperature regulation performed)	Not specified.
Müller et al.	No blood used	Icec-cold Ringers solution	After exsanguination, livers were flushed with ice-cold Ringers solution. The livers were then placed in the perfusion simulator.	2.5 to 4 hours cold ischemia time (mean 3 hours and 24 minutes)	Sub-normothermic. Ringers solution cooled before perfusion, but temperature not regulated during perfusion.	Not specified
Risse et al.	No blood used	Substrate enriched Krebs Henseleiit solution at 37 °C , 100% oxygenation	Before removing the livers, they were rinsed with cold Ringer's solution (5°C, 200 mL/kg body weight, Ringer Braun). After ischemia time, the organ was stored in warm Ringer's solution, now at 37 °C. After that, two hours of reperfusion followed. The organs were transported to the MRI while lowering the organ temperature to 5 °C.	24 hours	Normothermic	2 hours
Singh et al.	Human blood; time-expired, pH corrected (with Sodium Bicarbonate - NaHCO3 8.4% w/v), O-positive whole blood	No other perfusate used	After cannulation of the HA and PV, livers are flushed through both cannulas with 2 L of cold (4 C), heparinised UW (University of Wisconsin–Belzer UWVR Bridge to Life, Columbia, South Carolina) solution (5000 IU of heparin per litre of University of Wisconsin Solution-UW). After that, livers are transported in cold storage (4 °C).	Not specified	Normothermic (mean resulting liver temperature was 35.3 °C (SD ±0.35) and 35.6 °C)	30 minutes
Thompson et al.	No blood used	0.9% saline solution	After explantation and cannulation, the livers were transported to the lab within an hour. There, they were submerged in a 0.9% saline solution bath for drainage out of the IVC. The PV and HA were connected to the pump system and perfused with normothermic heparinized saline for one hour. Then, flushing with 10% neutral buffered formalin (Fisher Scientific/Acros, Waltham, MA) solution through the PV and HA followed for 15 minutes. For fixation, the HA PV and IVC were clamped off and the livers were submerged in formalin solution. To complete fixation, the livers were stored in a sealed container and left to fix for 5 days. To prepare laboratory perfusion experiments, fixed livers were removed from the fixation solution and placed in a 0.9% saline bath.	1 hour	Sub-normothermic (room temperature, no temperature regulation performed)	Not specified
van Leeuwen et al.	No blood used	Normothermic perfusion; clinical NMP solution containing a hemoglobin-based oxygen carrier Hypothermic perfusion; University of Wisconsin Machine Perfusion solution (Bridge to Life, London, UK)	The superrapid donor organ procurement method described by van Rijn et al. was follwed: After death and a very short ischemic time of 5 minutes, the aorta was flushed using at least 4L of ice cold (0-4°Belzer University of Wisconsin Cold Storage Solution (UW, BridgetoLife, London, UK) with 50000 U of heparin added. An extra flush was started before packing and transportation. Upon arrival, the hepatic artery was dissected further and cannulated. A final flush of the entire liver was performed with 1L of Belzer MPS®UW Machine Perfusion Solution(UW MPS) (Bridge to Life).	5 minutes	Normothermic (2 livers) and hypothermic (10-12 °C, 3 livers)	4 hours (specified for normothermic perfused liver, other livers perfused for at least one hour)
Zhao et al.	Autologous blood with 10000 U/L heparin	Perfusate consisting of 7 g/L sodium chloride, 0.3 g/L potassium chloride, 0.2 g/L calcium chloride, 3.1 g/L sodium citrate, 30 g/L 6% hydroxyethyl starch, 34 g/L mannitol and 3000 IU heparin	Livers were immersed in in heparinized perfusate and stored in a box with autologous blood at 4°C for 2-4 hours.	Warm ischemia time was kept within 30 min.	Hypothermic (20 °C in water bath)	First, perfusion using heparinised perfusate (duration not specified). After that, the livers were perfused using blood for 3 hours.

Table A1: Overview of the perfusion parameters described in the papers included in the systematic literature review. These parameters include perfusate, storage and preparation steps, perfusion temperature, and perfusion time.

Authors	Pump type	Oxygenator type	Heat exchanger integration	Additional elements of perfusion system
Alzaraa et al.	The system makes use of an automatic centrifugal pump, no further details presented in this paper. Previous study (Gravante et al.) mentions that an extracorporeal circuit (Medtronic Inc., Minneapolis, MN) is used, which contains a centrifugal pump	Integrated into Medtronic system according to Gravante et al.	Integrated in oxygenator according to Gravante et al.	The pump is used to provide arterial flow and pressure to the liver. A separate blood reservoir is used to simulate venous flow and pressure in a separate circuit. No further details presented in this paper.
Averkiou et al.	See Izamis et al. Same system and pump used here.	See Izamis et al. Same system and and oxygenator used here.	Not used (see Izamis et al.)	The same reservoir, flow loop, flow meters and manometers as used by Izamis et al. are used. The previously described bubble trap has not been used.
Bu et al.	A standard extracorporeal circulation unit (Polystan, Copenhagen, Denmark) was used for perfusion. Three mono-headed roller pumps are integrated into this system, tracking liver outflow through the inferior vena cava and flow perfusate from the reservoir to the liver via the HA and PV respectively	Not used	Separate heat exchanger used, type not specified.	Livers placed in a cylindrical tank with Perspex board and a thick Mylar membrane as an acoustic window for the liver. Another larger tank surrounds this tank with deionized water (20 ° C) for the HIFU beam. Perfusate in a separate reservoir, from which perfusate is pumped out using 2 pumps to the HA and PV, leading to the liver. The liver reservoir is actively drained using a third pump through the IVC. Pressure in the major hepatic vessels was measured using a PT-100 mutlichannel biological blood pressure sensor (Taimeng, Chengdu, China). Bile reservoir to catch bile from bile duct by negative pressure.
Civale et al.	The system described by Butler et al. Is used. They make use of a centrifugal pump (Medtronic BP50 Centrifugal Pump).	According to Butler et al. , a Jostra M15 Pediatric Membrane Oxygenator is used.	According to Butler et al., the oxygenator is attached to a separate heat exchanger (type not specified).	Before entering the liver, blood is pumped trough an oxygenator. The liver is placed in metal bowl and blood is pumped into the liver through the HA and PV cannulas (through lines of Medtronic, PVC, 1/4- and 3/16-inch ID tubing). The reservoir collects effluent perfusate, which is re-circulated into the system and collected separate blood reservoir (Jostra 800-ml soft shelled reservoir) Bile collected during perfusion in a separate reservoir. Additional nutrients are supplied using a IMED infusion pump. Pressure transducers (Baxter Triple Pressure Transducers), and flow probes (2 Medtronic DP 38P) are used to measure flow and pressure.
Gaffke et al.	10 or 30 ml artifical heart pumps, Berlin Heart Ag, Germany; driven by pneumatic driving units. (placed in MRI room, controlled by a pneumatic drive unit (989 Mbooster, Medizintechnik Dr. Heimes, Aachen, Germany) in the control room)	Three F7 hemoflow capillary dialysers (Fresenius Medical Care AG, Germany)	Temperature regulated in organ chamber instead of heat exchange in oxygenators or dialysers.	The liver is placed in a double-walled hemispherical organ chamber made of plexiglass (placed on a PVC frame), in which a normothermic temperature is kept. Blood is pumped into the HA and PV trrough the heart pumps and flow is regulated. Before flowing into the liver, perfusate is oxygenated in the dialyzers (although description is unclear due to translation). All pumps and dialysers are MR compatible and placed near the MR scanner. 8 meters of tubing (Fresenius Medical Care AG, Germany) is used to reach the driving system from the MR scanner. This length can be shortened if the driving system is placed on the 0.5mT control line.
Hildebrand et al.	Continuous flow perfusion pump (system pump, Radionics Inc., Burlington, MA)	Not used	Not used	Only perfusion through the PV, which was connected to an elastic tube. The other end of the tube was connected to the inferior vena cava, creating a closed loop. The pump was connected to this tube to pump blood through the liver. Perfusate could also drain from the HA.
Izamis et al.	Peristaltic pump (Masterflex L/S Digital Drive 600 rpm, Cole–Parmer)	Affinity NT, Medtronic, Minneapolis, MN, USA	Temperature not regulated	The liver was placed in an open reservoir (plastic container with permeable nylon membrane (B004QZ9XKI, Small Parts, Logansport, IN, USA) to suspend the liver). Perfusate is pumped into the circuit through tubing (24G and 16G tubing (EW-96410-24 and SI-96410-16, Cole–Parmer, Vernon Hills, IL, USA). Once oxygenated, the tubing is split into two, creating two separate flows for the PV and HA. For both PV and HA flow, flow meters (EW-32461-44 and EW-32460-40, Cole–Parmer) and manometers to measure flow rate and pressure. After reaching the liver, the effluent is allowed to flow freely from the liver's vena cava, closing off the system. A 500-mL bubble trap (Radnoti, Monrovia, CA, USA) is used to filter out bubbles and dampen pulsatile flow.
Keravnou et al.	See Izamis et al. Same system and pump used here.	See Izamis et al. Same system and and oxygenator used here.	Not used (see Izamis et al.)	Same reservoir, flow loop, flow meters and manometers as used by Izamis et al. The previously described bubble trap has not been used.
Liu et al.	LifePort liver transporter (Organ Recovery Systems, Zaventem, Belgium) is used. Based on a manual from a silmilar system for kidney perfusion by the same company, they make use of a roller pump for pulsatile blood flow.	LifePort liver transporter most likely has an integrated oxygenator, however, this is unclear from the manual.	Oxygenator most likely has an integrated heat exchanger, however, this is unclear from the manual.	No elements added to the system, the casette that the liver is placed in is MR-compatible.
Lorton et al.	The system used here is described by Buchs et al., originally developed for kidney perfusion. It makes use of a pulsatile pump (adapted from a peristaltic 'Arthopump'), which is controlled by pressure and is driven by the oxygen flow. The pump starts when diastolic pressure is reached, stopping again when systolic pressure has been reached in a controlled feedback loop.	According to Buchs et al. the system is driven by oxygen, making use of a large oxygen tank. The residual oxygen at the outflow of the pump is used to oxygenate the perfusate, using a 'porous' filter (type not specified).	Not used, temperature of organ regulated by the cooling box according to Buchs et al.	An 'umbilical cord' consisting of two 7m lines of tubing (one for oxygen driver and one for pressure control) connects the driving system in the control room to the organ in the MR scanner. The organ is placed in the perfusion module, which consist of a circular reservoir in a 40cm cube-shaped cooling box.
Müller et al.	The system is based on a custom-designed respiratory liver motion simulation device, described in previous research. Biomedicus 540 Centrifugal Pump (Medtronic, Minneapolis, MN) used for continuous venous flow. HABP100 (Instech Laboratories, Inc.,PlymouthMeeting, PA) for pulsatile arterial flow.	Not used	Not used	Flowmeters (CardioMed CM 1005; Medi-Stim A/S, Oslo, Norway) used to measure HA and PV flow rate in both perfused and nonperfused livers. No further system details described.
Risse et al.	4 pumps are used; 2 for HA and PV flow, 2 for temperature regulation (see 'Additonal elements' for further details). Peristaltic pumps (505Du/RL with pump head 501RL, Watson-Marlow, Falmouth, GB)	Two Maxima Puls PRF Oxygenators, Medtronic Inc., Minneapolis, USA	A separate heat exchanger is used to regulate perfusate temperature, coupled to thermostat (F6, Haake, Karlsruhe). An extra thermostat measures regulates the temperature in the liver reservoir directly.	The liver is placed on a net and submerged in a perfusate bath made of Plexiglass. This is especially developed for use in MRI and contains a water-filled double wall for maintaining liver temperature. Perfusate is collected in a reservoir, pumped through oxygenators and supplied to the liver in two separate circuits for HA and PV flow, using two of the pumps. Effluent perfusate can flow freely into the liver reservoir out of the IVC, a third pump is used to actively pump it back into the perfusate reservoir. A fourth pump pumps perfusate through a heat exchanger. DPL-060PKS (Kobold Messring, Hofheim/Taunus) sensors were used to measure flow. Piezoresistive pressure transducers (0-0.35 barm RS components, Mörfelden-Walldorf) were used to measure pressure.
Singh et al.	Purpose-bult dual roller pump (Watson Marlow, Cornwall, UK) perfusion system, makes use of three of these pumps.	Capiox TherumoVR SX 10, Therumo Cardiovascular Systems, Terumo Shibuya, Tokyo, Japan	Integrated into oxygenator	Separate reservoirs for liver and blood are used. Bile is collected as well in a separate reservoir. Blood is pumped through an oxygenator and back into the reservoir via one loop of tubing. Two other pumps pump oxygenated blood into the liver through the HA and PV cannula's respectively and the effluent blood flows back from the liver into the reservoir.
Thompson et al.	Venous flow: Bio-Medicus® 560 (Medtronic Inc., Minneapolis, MN) perfusion circuit. Used for single and double input perfusion for continuous PV flow. Arterial flow: Masterflex® L/S™ peristaltic pump (Cole-Parmer Instrument Co., Bunker, CT). Used for double-input perfusion for pulsatile HA flow.	Not used	Not used	Flow and pressure transducers (types not specified) added into the system to measure portal venous flow rate. A body-shaped CT compatible acrylic container (30x27 cm) was used as a liver reservoir, where the phantom is submerged in perfusate. The reservoir was placed on a raised platform to allow drainage of perfusate through the IVC.
van Leeuwen et al.	The XVIVO Liver Assist system is used. Integrated in this system are two rotary pumps (Deltastream DP2, Medos AG, Stolberg, Germany), one for pulsatile arterial flow, one for continuous venous flow.	Oxygenators (Medos AG, Stolberg, Germany), size used in this study not specified.	Heat exhangers integrated into oxygenators	The whole system is integrated into a mobile trolley system with a table. In a sink-like structure in the table, there is the liver reservoir, where the liver lies on a net and can be submerged in perfusate. Sensors for measuring temperautre, flow and pressure are integrated into the system (and displayed on a monitor) and results are used to control the pumps to reach physiological flow and pressure values.
Zhao et al.	See Bu et al. The same extracorporeal circulation unit (Polystan, Copenhagen, Denmark) was used with the same pumps.	Not used (see Bu et al.)	Not used (see Bu et al.)	See Bu et al. The same extracorporeal circulation unit (Polystan, Copenhagen, Denmark) was used as used by Bu et al. with the same additional elements.

Table A2: Overview of the perfusion setups described in the papers included in the systematic literature review. Pumps, oxygenators, heat exchangers, and other elements such as reservoirs and sensors are described.

Authors	Imaging method	Details
Alzaraa et al.	Contrast-Enhanced Ultrasound Imaging (CEUS)	Imaging performed LOGIQ 7 US machine (GE Healthcare, Chalfont St Giles, UK)With Sonovue contrast agent. Wash-in wash-out sequences and modes described. Convex transducer 2-5 MHz. Imaging was performed after 1 and 4h of perfusion.
Averkiou et al.	Dynamic Contrast- Enhanced Ultrasound Imaging (DCEUS)	A similar CEUS setup was used as described by Izamis et al. with several (therapeutic and imaging) transducers operating around 3.1 MHz. The same microbubbles as described in Keravnou et al. were been used for contrast, allowing for qualitative and quantitative analysis. The effect of microbuble concentration on quantification parameters under constant flow was investigated using 8 different microbubble solutions. The effects of varying HA and PV flow rates on these parameters were investigated as well by varying HA and PV flow rates (see 'Flow waveform and boundaries') in different sequences.
Bu et al.	Color Doppler flow imaging and pulse wave imaging	HIFU was performed and guided using a 3.5-MHz color Doppler diagnostic ultrasound device (Esaote). In addition to this, every thirty minutes of perfusion, coaxial US imaging was performed using a 12-MHz probe (Esaote, Genoa, Italy). Of these imaging results, flow velocities could then be derived. Barrier location of necrosis regions after HIFU exposure were investigated as well and compared to histological results.
Civale et al.	Backscatter temperature imaging using ultrasound. High intensity focussed ultrasound (HIFU)	HIFU was generated using different transducers (Imasonic, Besanc¸on, France) all operating around 1.7 MHz. Ultrasound backscatter temperature imaging was applied using a Zonare ultrasound scanner (Z.One, Zonare Medical Systems, Mountain View, CA, USA). A curvilinear probe operating at 2.8 MHz was used to image HIFU focal regions. A backscatter temperature imaging algorithm was used on this data to obtain temperature data at the ablation zones. These temperatures were compared to thos emeasured using needle thermocouples inserted in the tissue.
Gaffke et al.	Magnetic resonance imaging (MRI)	The Siemens Symphony 1.5T with quantum gradient coil (Siemens, Erlangen Germany) was used to perform MRI scans. To simulate experimental interventions in-vivo, MR-supported punctures were perfomed using an MR-compatible titanium needle and catheter system (AnaKat, Berlin, Germany) and thermal ablation was performed using a laser applicator (e.g. Huettinger, Umkirch, Germany. Several sequences were used, among which T1-weighted gradient echo sequences for investigating thermal effects of ablation. Different types of T1 FLASH (fast low angle shot) sequences were also used for anatomical orientation and angiography scans (with Magnevist used as a contrast agent).
Hildebrand et al.	Ultrasound-guided radiofrequency ablation	An examination of the whole liver was done using a high resolution US probe (B&K Medical 8566) and tumor-mimicks were localised. After that, radio frequency ablation (RITA Medical System Inc.) was performed under US-guidance in a laparoscopic device (B&K Medical 8566, Denmark). Duplex ultrasound was also performed for imaging of liver perfusion (most likely using the same device, but this was not specified).
Izamis et al.	Dynamic Contrast- Enhanced Ultrasound Imaging (DCEUS)	The iU22 ultrasound scanner (Philips Healthcare, Bothell, WA, USA) is positioned alongside the organ, and different probes (3.1 and 6.3 MHz) are used to image the micro- and macrovascularity of the liver, scanning from a fixed location over time. This was done using 0.2 mL of Sono-Vue contrast agent (Bracco, Milan, Italy) dministered every hour trough HA and PV with intermittent scanning. To characterize the flow through the vasculature, time–intensity curves are created. Other probes (3.9 and 8.2 MHz) provided qualitative evaluation of the perfusion throughout the organ, eliminating microbubbles in the process.
Keravnou et al.	Contrast- Enhanced Ultrasound Imaging (DCEUS)	A similar CEUS setup was used as described by Izamis et al. with several (therapeutic and imaging) transducers operating around 1 MHz. Custum-made microbubbles were injected, which were driven and cavitated by the therapeutic transducer. Imaging the resulting regions of interest after cavitation allowed for both qualitative and quantification of flow in well-perfused regions of interest after time-intensity curve calculation. Livers were divided into 4 groups. In each group, different acoustic pressures (1.7-4 MPa) and number of cycles (1000 or 20) were applied. The influence on microvascular perfusion and microbubble cavitation activity was assessed.
Liu et al.	Magnetic resonance imaging (MRI)	MRI type or magnetic field strength not specified. Diffusion weigthed MRI scans were applied every 12 hours for hypothermically perfused livers and every 30 minutes for the other livers. Perfusion imaging witht a gadolimium-based contrast agent was used to visualise the hepatic circulation. Apparent diffusion coefficients were analysed for these groups of livers.
Lorton et al.	High-intensity focussed ultrasound (HIFU, both continuous-wave and pulsed) under ultrasound-guidance. Magnetic resonance imaging (MRI) was used to guide the HIFU beams.	HIFU was generated by an MR-compatible phased array transducer (Imasonic, Besanc¸on, France) at frequency of 1031 kHz. This signal was used to ablate tissue in the perfused organs. PFOB-FTAC micro-droplet sonosensitizers were injected as well. MR data was acquired using a 3 T whole body MR scanner (Prisma Fit, Siemens, Erlangen, Germany) . First, 3D T1-weighted scans (T1-VIBE) were performed for HIFU focal point positioning, after which the ablation described above could take place. MR thermometry was applied to monitor temperature changes in the tissue. Finally, assessment of perfusion was done using dynamic contrast enhanced (DCE) perfusion scans with 0.5 mmol of Dotarem as a contrast agent.
Müller et al.	Computed tomography (CT)	Different liver sections were visualised by placing a catheter (Logicath AgTive; Medex Medical Inc., Rossendale, Lancashier,UK) guided by fluoroscopy (Siemens Siremobil Compact; Siemens Healthcare, Erlangen, Germany). Before scanning, flows were calibrated for 1 0 minutes. A Somatom 16 multi-slice scanner (Siemens, Erlangen, Germany) was used to perform helical CT scans. 80 mL Ultravist (Schering, Berlin, Germany) was added for contrast-enhanced scans. This contrast agent was also adminisered to the catheter mentioned above to obtain information of pefusate flow in the different sections. Protocols for liver volumetric assays and liver plasticization (described in previous studies) were followed using 3D CT reconstructions and validated with water displacement methods.
Risse et al.	Magnetic resonance imaging (MRI)	MRI (MAGNETOM Vision, Siemens, Erlangen) is used to take T1 and T2-weighted scans to investigate liver anatomy. Therafter, contrast-enhanced perfusion scans were performed using T2*-echo planar imaging (EPI). For this, 0.01 mmol/kg body weight of Magnevist (Schering, Berlin) was used as a contrast agent, supplied to HA and PV separately. Of these results, time-intensity curves were plotted and from these signal intensities (before and after contrast), the local contrast medium concentration was be calculated for several segmented regions of interest. This was done for both HA and PV flow.
Singh et al.	Microwave ablation under ultrasound-guidance performed during perfusion. Laser Doppler flowmetry also applied during perfusion. Magnetic resonance imaging applied as well, but not during perfusion.	Microwave ablation under ultrasound guidance (Hitachi EUB 6500VR Hitachi, Tokyo, Japan) was performed on perfused and non-perfused livers. A dual-channel Doppler Flowmetry (LDF) system (DRT4; Moor Instruments, Ltd, Axminister, UK) with a laser source and a multi-detector integrating laser Doppler probe (DP7; Moor Instruments, Ltd, Axminster, UK) was used in addition to this to investigate the surface microcirculation of blood in the ex-vivo perfused livers. This was done at locations away from the ablation zones.
Thompson et al.	Dynamic contrast-enhanced computed tomography imaging	Single input imaging with only PV flow: scans were performed using a 128-slice, dual-source, multidetector CT scanner (Definition Flash, Siemens Healthcare, Forcheim, Germany). Several input flow rates were tested during this imaging phase. Scans were taken at different contrast volumes and injection speeds. At each flow rate, Dual input imaging with PV and HA flow: scans were performed using a 128-slice, dual-source, multidetector CT scanner (Definition Flash, Siemens Healthcare, Forcheim, Germany). With set flow rates for HA and PV, 4 mL of contrast agent in both HA and PV were injected, separated by 10 seconds. CT scanning was initiated 6 seconds thereafter.
van Leeuwen et al.	Doppler ultrasound	Doppler Ultrasound (machine not specified) was performed on machine perfused livers to confirm proper catheter placement and to qualitatively image flow in and out of the livers.
Zhao et al.	High-intensity focussed ultrasound (HIFU, both continuous-wave and pulsed) under ultrasound-guidance.	HIFU was performed using a therapeutic system (Model JC200, Chongqing Haifu Medical Technology Co., Ltd., Chongqing, China). This was done under different PV flow rates (see 'Flow boundaries / Portal vein') in both continuous-wave and pulsed mode. During HIFU exposure, caviation emission was recorded to detect the acoustic emission at the focus of the transducer. A 3.5-MHz colour Doppler diagnostic ultrasound device (Esaote, Genoa, Italy), functioning at 0.9 MHz, was used for US guidance and flow imaging.

Table A3: Overview of the imaging experiments described in the papers included in the systematic literature review.

Authors	Conclusions on organ viability	Additional remarks/Progress in perfusion system development
Alzaraa et al.	Livers deemed viable after 6 hours of perfusion. No dramatic changes of flow rate, pressure and oxygen saturation values. Bile production increased after 1 hour, reached a peak at 2 hours and reached a steady production after that. However, DCEUS showed perfusion defects after 4 hours of machine perfusion.	The authors refer to previous research (Gravante et al.) for a description of the perfusion system. They concluded that contrast-enhanced ultrasound is a useful tool for identifying areas of the liver that are hypoperfused in our ex vivo porcine liver model. The model proved to be a useful platform for testing US-based methods. DCEUS showed additional perfusion information.
Averkiou et al.	Hepatic stability was ascertained with measurements of bile-production, oxygen consumption and sustained vascular perfusion (similar to Izamis et al.), however, no specific results are presented.	This study is a continuation of the studies by Izamis et al. and Keravnou et al., so the same system has been used. Few results are presented regarding the standard hemodynamic and liver viability testing, but system performance can be compared to what is described by Izamis et al. The results on DCEUS imaging and correpsonding perfusion characteristic relations are presented. These results demonstrate that induced flow rate changes can be measured precisely by DCEUS, confirming the suitability of DCEUS for monitoring flow
Bu et al.	Based on histology samples, parenchymal morphology and architecture of the livers remained overall intact during the 3 hours of perfusion. Bile secretion by the liver continuously increased during the 3 hours of perfusion, indicating proper bile production and functionality of the liver	The authors were able to use this system to test out HIFU-techniques. The flow rate in the major hepatic vessels of the perfusion system has been proven to be a strong predictor of total energy consumption after ablation. The total energy consumption decreases significantly with a decrease in flow rate. Whether measured flow rate also correlates similarly with overall liver viability is an interesting topic for future research.
Civale et al.	No conclusions presented on organ viability in this study. However, the study by Butler et al. Demonstrates the same system under similar circumstances and they deemed perfused livers viable even after 24 hours of perfusion based on oxygen consumption and histology results.	The system used in this study was described by Butler et al. Results on liver viability presented in this previous research can be applied here, since livers were perfused under similar circumstances. A system is described that can perfuse organs for up to 24 hours (or possibly up to 72 hours described by Bu et al.) at normothermic temperatures. The addition of nutrients into the system might have contributed to this as well. Nonetheless, this system could perfuse of livers for long periods of time, allowing for a stable platform for different types of ultrasound imaging. The presented levels of pH, glucose, and partial gas pressures give a good indication on how these parameters should stabilise over time of perfusion.
Gaffke et al.	Liver deamed viable over 4 hours of perfusion. Until the end of perfusion, oxygen consumption and bile secretion wre detectable and remained within the tolerance limits. The temperature also remained stable during perfusion.	This study demonstrates the use of their MR-compatible system for perfusing porcine livers while performing experiments such as thermal ablation under the MRI scanner. Although no clear description of the system settings are given in this paper, previous research presents indications of flow and pressure boundaries that can be reached with such a system. The driving system used in this study could be placed on the 0.5mT control line for use in the MRI. It is definately worth trying out whether this is also possible with the system built for this thesis. It would also be interesting to explore the use of dialyzers in perfusion systems.
Hildebrand et al.	No viability testing performed. Authors deemded perfusion through the PV to be feasible during the entire operation and US results indicated sufficient circulation of perfusate throughout the livers.	The setup used is quite basic, consisting of a cannulated liver connected to a continuous flow pump using tubing. No flow rate or pressure values are described in this study. The imaging experiments performed on this system are described clearly. The autors were able to conclude proper perfusion based on imaging results. This system has thus been proven to be a useful platform for imaging exper. However, no clear conclusions can be drawn on how viable the livers were based on the results of this paper.
Izamis et al.	Livers deemed stable after 3 hours of perfusion. Based on histology samples, liver function decreased after static cold storage and improved within 2 hours of flishing with cold storage solution. Bile production was also deemed stable after 1 hour of perfusion. DCEUS imaging showed homogeneous perfusion in both the PV and HA within an hour of machine perfusion. Thrombi could be found on these results that resolved over time.	A ratl liver machine perfusion system was scaled up to be able to perfuse pig livers. The findings of this study suggest that this stable system can keep livers viable for longer than the 3 hours of time the system was demonstrated for. The authours recommend performing dynamic contrast imaging of any kind (US demonstrated here) for the qualitative and quantitative assessment of perfusion. These results provide a valuable imaging protocol to be followed besides routine organ viability testing.
Keravnou et al.	Hepatic stability was ascertained with measurements of bile-production, oxygen consumption and sustained vascular perfusion (similar to Izamis et al.), however, no specific results are presented.	This study is a continuation of the study by Izamis et al., so the same system has been used. Few results are presented regarding the standard hemodynamic and liver viability testing, but system performance can be compared to what is described by Izamis et al. Using this system, the authors were able to induce perfusion changes by using mircobubbles under different US-parameters and those changes have been visualised using ultrasound imaging. Further research is recommended on the benefits of longer pulses in US-controlled drug delivery. This research benefits from isolated organ perfusion systems such as this one.
Liu et al.	Due to warm ischemia time, livers were considered non-viable. All livers showed severe morphological alterations and high transaminase release.	No flow rate or pressure values are described in this study. The livers were also considered non-viable after perfusion. The authors describe the use of an MR-compatible system (not yet approved for clinical use) and were able to conclude that apparent diffusion coefficient from MRI is a sensitive indicator of hepatic ischemia in-vivo. However, this conclusion cannot be drawn from these results when applied to ex-vivo perfused livers.
Lorton et al.	Not specified. Buchs et al. Describes that perfusion can be sustained for up to 24h.	The system used is one described by Buchs et al., which is a pressure-controlled system for kidney perfusion. Few details are presented on how this system was adapted to perfuse livers. Organ viability was not tested either, as they mainly focussed on the experimental tissue-mimicking model that was used besides ex-vivo kidneys and livers. Still, the authors found this system to be suitable for testing MR-guided HIFU methods and similar applications.
Müller et al.	No viability testing performed.	This study presents the use of a perfusion system at a variety of flow rates, allowing for liver volumetry measurements using CT imaging. The system itself is not described in great detail, since the original system it was based on was used to simulate respiratory motion (and not necessarily made to keep livers viable for a long time). Liver viability has not been studied, however, the authors deemed the simulator to be a good device for studies in liver perfusion and could surve as a platform for practicing surgical skills on livers.
Risse et al.	Livers were stable up until 1.5 hours of perfusion in the setup. After that, significant edema and with that an increase in liver volume were visible, indicating a reduced liver function.	Few results are presented in this study regarding the used settings (or measured results of) flow rate and pressure. However, a clear description of the MR-compatible system is given. It is interesting to see how temperature is regulated in both the liver reservoir and perfusate reservoir. This system Regional perfusion defects in the livers could be observed from MRI results. The described method of segmenting the liver and calculating time-intensity curves for each segment is very useful for evaluating perfusion using imaging.
Singh et al.	Based on Laser Doppler flowmetry results, the microcirculation of the perfused livers increased progressively due to perfusion (from a mean pre-ablation flux of 14.09 ± 1.8 to a post-ablation flux of 17.23 ± 3.3 arbitrary perfusion units ($p = 0.006$)). All livers had an increase in microcirculation as a result of re-perfusion and re-warming ($p = 0.34$). Additionally, bile continued to flow during perfusion, indicating proper liver function.	The same flow rates were chosen for both the HA and PV based on a paper by Siriwardana et al. This set of preliminary experiments was designed to select the perfusion flow rate which resulted in optimum peripheral tissue perfusion with minimal pressure induced parenchymal damage. Using perfused and non-perfused groups, this research showed that pathogenesis of ablation zone extension is not dependent on blood flow. No remarkable conclusions are presented on the functioning of the perfusion system, althoug the design of the system with a separate loop for oxygenation is interesting for future research.
Thompson et al.	Liver was fixed to create a phantom, so viability not validated. However, based on histology results, hepatic architecture, HA and PV and endothelial cell structure all intact and recognizable in the fixed state. There was no evidence of hepatocyte necrosis.	This paper cannot be compared to other papers regarding liver viability, as a fixative has been used. This paper was mainly focussed on developing the porcine liver phantom that can be used for a long period of time. Still, this paper does demonstrates a broad range of flow rates of perfusion using a self-designed system. Reproducible dynamic contrast-enhanced CT images taken during perfusion have confirmed that this system is imaging compatible. Such a system could also keep a liver viable with the correct perfusate.
van Leeuwen et al.	All livers were viable after perfusion. Some specific results for the different types of perfusion are shown below: Hypothermic perfused livers: No results on viability testing presented. Normothermic perfused livers: Lactate and pH in the perfusate stabilised within 2 hours of perfusion. Biliary pH stabilized after 1 hour. Left lateral split perfused livers: perfusate lactate and pH stabilised within one hour.	This study presents a unique method of cannulating livers, supplying (portal)venous flow to the liver via the umbilical vein. Even the left lateral split perfusion method, in which only one part of the liver is perfused, shows feasible results regarding umbilical vein perfusion. The clinical Liver Assist system by XVIVO provides a very stable platform for perfusing livers for studies involving ultrasound imaging.
Zhao et al.	Based on pressure measurements and Colour Doppler flow imaging, intra-hepatic vessels were deamed regular. The pressure and flow velocities remained stable over 3 hours of perfusion. Histology results showed limited local monocyte infiltration. Parenchymal morphology and architecture of the liver also remained stable during perfusion. Moreover, bile production remained constant. Based on all of these parameters, the authors confirmed a normal organ function.	This seems to be a continuation of the study by Bu et al. (as a very similar system has been used and some authors correspond), although no reference to that study has been made. The authors were able to investigate the effect of flow rate on HIFU results using this perfusion-model. No additonal conclusions on the functioning of the perfusion system have been given.

Table A4: Overview of the conclusions drawn from the papers included in the systematic literature review.

A.3 Documentation of requirements

A.3.1 User requirements specification

User requirements for an isolated liver perfusion system to be used in magnetic resonance imaging

Revision	Latest version: 15.11.2022
Document gets invalid	_
This document replaces the document specified above. With the release and output of this document, all previous documents become invalid. Invalid originals and copies must be marked as "invalid" by the respective owner, sorted out and replaced by the valid version	

Status	Date	Name/Area	Key	Signature
created	15.11.2022	M. Krommendijk	MK	
checked				
approved				
Template	EOST0904_TEM_QM-docs_210716JAS.dotx			

Content **page**

1 Task definition

The aim is to develop a system that can perfuse an isolated porcine liver for 4 to 6 hours. A physiologically relevant environment for the liver should be provided by the system to keep the liver viable and functional during perfusion. The system should also be designed in such a way that perfusion can take place under an MRI scanner.

2 Terms and abbreviations

For terms, abbreviations and symbols not listed here, please refer to the document glossary.

2.1 Terms

Indication

The reason to carry out medical therapy, after assessing the possible risk

Oxygenator

Blood gas exchanger (oxygen and carbon dioxide).

Heat exchanger

Integrated exchanger of heat in oxygenator

Disposable

Item (i.e. tubing, oxygenator or pump head) meant for single use in clinical settings . In this case, items can be re-used when no physical damage is visible.

2.2 Abbreviations

- **O_2**: oxygen
- **CO_2**: carbon dioxide
- **URS:** user requirement specification
- **DRS**: design requirement specification
- **FU:** functional unit
- **N/A:** not applicable

3 Device specifications

The device under development shall include the following functional units:

- FU 1: Liver reservoir
- FU 2: Blood tubing
- FU 3: Arterial pump
- FU 4: Venous pump
- FU 5: Arterial oxygenator
- FU 6: Venous oxygenator

Not part of the development project, but still subject of consideration because of interfaces:

- Arterial pump console (compatible with chosen arterial pump)
- Venous pump console (compatible with chosen venous pump)
- Flow sensors and monitor
- Pressure sensors and monitor

- Heat regulator
- Gas supply
- Additive supply

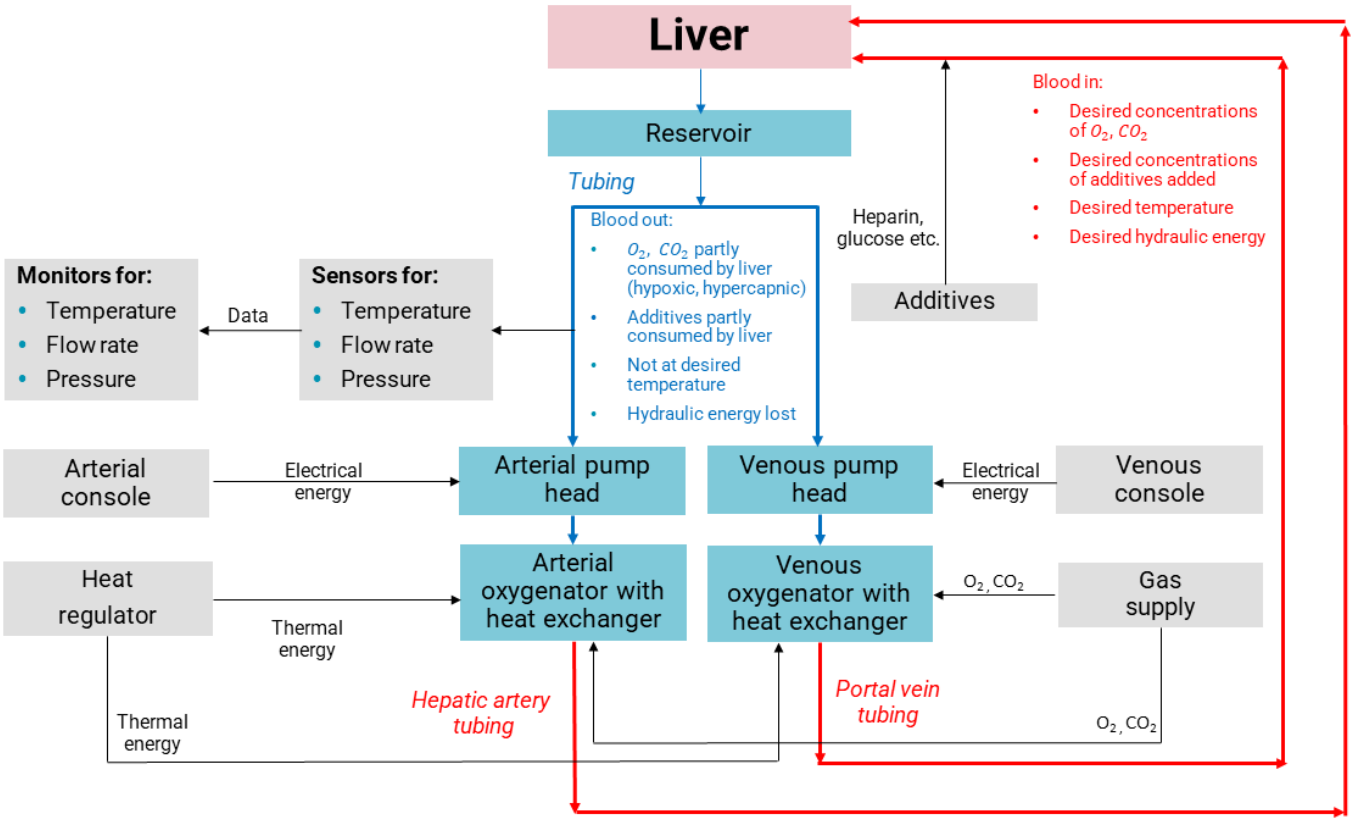

Figure 3-1: System schematic with interfaces of the isolated liver perfusion system

3.1 Intended use, indication

Description of requirements	URS-ID
The product shall be intended for the perfusion of porcine livers.	3.1-010
The product shall be designed for porcine livers weighing 1 to 3 kg.	3.1-020
The product shall allow for different perfusates to be used, such as isotonic saline solution and porcine blood.	3.1-030
The product should be useable as a perfusion system for a porcine liver in an MRI scanner	3.1-040
N/A	3.1-050

3.2 Classification and duration of use

Description of requirements	URS-ID
The product shall be authorised for the perfusion of on porcine livers for several consecutive hours of experimentation	3.2-010

3.3 Shelf life

Description of requirements	URS-ID
The shelf life of the driving system of the product shall be several years	3.3-010
The shelf life of the disposables shall be two weeks up to one month	3.3-020

4 Prerequisites for use

Description of requirements	URS-ID
The operating temperature should be close to a normothermic temperature, 35 to 39 °C	4.0-010
N./A	4.0-020
N./A	4.0-030

5 Application and users

5.1 Application process

Description of requirements	URS-ID
The product shall be able to be disassembled easily	5.1-010
The interchangeability of the following components shall be realized: Tubing and connectors, pump heads, oxygenators, reservoirs,sensors	5.1-020
N/A	5.1-030

5.2 Users

Description of requirements	URS-ID
The intended user is regarded as an engineering student, medical student or medical professional	5.2-010
N/A.	5.2-020
The user must have knowledge on working with blood and animal organs before using the system	5.2-030
N/A	5.2-040

6 Device design

Description of requirements	URS-ID
The system must be mobile, suitable for transport within and between buildings	6.0-010
The driving system should be placed at a safe distance of the MRI scanner while the liver reservoir is placed in the MRI scanner	6.0-020
	6.0-030

6.1 Dimensions, Tolerances, Weight

Description of requirements	URS-ID
The system must fit through the doorways and passages along the path that the system will be transported.	6.1-010
The full system weight must allow fo the system be able to be pushed forward by one person	6.1-020
The product shall be easy to dismantle for cleaning and disposal.	6.1-030
N/A	

6.2 Materials

Description of requirements	URS-ID
The materials of the device that come into contact with the liver and/or blood shall be biocompatible.	6.2-010
The device materials shall be cleanable with water and/or saline solution.	6.2-020
All materials placed close to the MRI must not contain any ferromagnetic materials to avoid disturbance of the magnetic field.	6.2-030
All materials used shall be DEHP free.	6.2-040
All user contact materials shall be latex free for safety of the users.	6.2-050
N/A	6.2-060
N/A	

6.3 Connection techniques

Description of requirements	*URS-ID*
It shall be possible to replace the disposables during operation	6.3-010
N/A	6.3-020
N/A	6.3-030
Pump heads must connected to pump drives in a stable way	6.3-040
Tubing shall be connected with stable connectors	6.3-050
N/A	6.3-060

6.4 System design/structure

Description of requirements	URS-ID
The flow sequence when used as a liver perfusion system should be as follows: • Liver reservoir • Tubing (hepatic artery and portal vein) • Pump heads • Oxygenators • Tubing • Liver (into cannulas of hepatic artery and portal vein)	6.4-010
N/A	6.4-020

6.5 Cleaning/ Reprocessing/Sterilisation/Desinfection

Description of requirements	URS-ID
The driving system (a.k.a. pump heads) are to be disinfected	6.5-010
The blood-contact parts of the system are to be cleaned using a specified cleaning solution	6.5-020
N/A	6.5-030

6.6 Packaging

Description of requirements	URS-ID
Disposables are to be stored in their delivered packaging	6.6-010
N/A	6.6-020
N/A	6.6-030
N/A	6.6-040
N/A	6.6-050

7 Features/Functions

Description of requirements	URS-ID
Arterial blood flow such as in the hepatic artery is to be mimicked	7.0-010
Venous blood flow such as in the portal vein ist o be mimicked	7.0-020

Description of requirements	URS-ID
Blood is to be oxygenated	7.0-030
Flow rate and pressure shall be monitored during perfusion.	7.0-040

7.1 Functional unit 1: Liver reservoir

Description of requirements	URS-ID
The liver reservoir must fit a porcine liver	7.1-010
The liver reservoir must fit inside the bore of the MRI scanner	7.1-020
N/A	7.1-030

7.2 Functional unit 2: Blood tubing

Description of requirements	URS-ID
The tubing shall fit onto oxygenators and pump heads	7.1-010
The functional unit shall comply with the normative requirements.	7.1-020
N/A	7.1-030

7.3 Functional unit 3: Arterial pump

Description of requirements	URS-ID
The arterial pump should provide pulsatile arterial flow of blood mimicking that in the hepatic artery	7.3-010
The pump head or other parts that come in contact with perfusate shall be compatible for use with blood	7.3-020
The functional unit shall comply with the normative requirements.	7.3-030

7.4 Functional unit 4: Venous pump

Description of requirements	URS-ID
The venous pump should provide continuous venous flow of blood mimicking that in the portal vein	7.3-010
The pump head or other parts that come in contact with perfusate shall be compatible for use with blood	7.3-020
The functional unit shall comply with the normative requirements.	7.3-030

7.5 Functional unit 3: Arterial oxygenator

Description of requirements	URS-ID
The arterial oxygenator must be compatible with the specified arterial blood flow	7.3-010
The arterial oxygenator must have an integrated heat exchanger for temperature regulation	7.3-020
The functional unit shall comply with the normative requirements	7.3-030

7.6 Functional unit 6: Venous oxygenator

Description of requirements	URS-ID
The venous oxygenator must be compatible with the specified arterial blood flow	7.3-010
The venous oxygenator must have an integrated heat exchanger for temperature regulation	7.3-020
The functional unit shall comply with the normative requirements	7.3-030

8 Operation and control, interfaces

8.1 Interfaces system to periphery

Description of requirements	URS-ID
A clear interface to the pump control shall be available	8.1-010
A clear interface tot he sensor control shall be available	8.1-020
Pump heads must have interfaces for blood flow	8.1-030
Oxygenators must have interfaces for blood flow	8.1-040
Oxygenators must have interfaces for gas flow	8.1-050

8.2 Human-system interfaces

Description of requirements	URS-ID
The system shall be operable by a trained doctor/medical professional. N.A	8.2-010
N/A	

9 Provision of information by the manufacturer

Description of requirements	URS-ID
All information for the user of the system must be provided in English	9.0-010

9.1 Instructions for Use (IfU)

Description of requirements	URS-ID
Manuals must be provided by the manufacturers of all functional units and periphery units (if applicable)	9.1-010
N/A	9.1-020

9.2 Labeling primary product

Description of requirements	URS-ID
The labelling of the system must make it clear that the system belongs to the Liver-Twin project of the M3i group	9.2-010
The labelling must make it clear to the user how the system is to be assembled	9.2-020

9.3 Labeling Packaging

Description of requirements	URS-ID
The labelling of the packaging must make it clear that the system belongs to the Liver-Twin project of the M3i group	9.3-010
N/A	9.3-020

9.4 Training documents

Description of requirements	URS-ID
N/A	9.4-010

10 Storage and transport conditions

Description of requirements	URS-ID
The permissible ambient temperature for storage shall be 18 °C to 24 °C	10.0-010
The permissible ambient temperature for transport shall be 10 °C to 30°C	10.0-020
N/A	10.0-030

11 External specifications

Description of requirements	URS-ID
Re-use of disposables (pump heads, oxygenators, tubing) is only allowed if no physical damage of the components is visible.	11.0-010

11.1 Economic demands

Description of requirements	URS-ID
N/A	11.1-010

11.2 Legal provisions/standards

Description of requirements	URS-ID
N/A	11.2-010
N/A	11.2-020
N/A	11.2-030

12 Production and further treatment

Description of requirements	URS-ID
N/A	12.0-010

12.1 Production

Description of requirements	URS-ID
N/A	12.1-010

13 Maintenance/service

Description of requirements	URS-ID
Maintainance of individual functional units and periferies should occur conform their respective manuals	13.0-010

14 Disposal

Description of requirements	URS-ID
The disposal of the secondary packaging of the product shall be carried out via the local recycling system.	14.0-010
The disposal of blood-bearing parts of the product shall be carried out via the disposal routes of biologically contaminated materials.	14.0-020
Disposable parts of the be disposed via 30L SZA-WIVA containers for biological waste	14.0-030

15 Clarification of conflicting claims

Conflict	Affected URS-IDs	Solution
N/A		
N/A		

16 Notes and applicable documents

16.1 Applicable documents

- URS is coupled to corresponding DRS

17 Document revision status

Create date	Revision	Author	Brief description of the change
25.11.2021	00	J. Arens	First version of template

Create date	Revision	Author	Brief description of the change
15.11.2022	01	M. Krommendijk	Finished draft for master thesis

18 Attachments

A.3.2 Design requirements specification

Design requirements for an isolated liver perfusion system to be used in magnetic resonance imaging

Revision	Latest version: 15.11.2022
Document gets invalid	_
This document replaces the document specified above. With the release and output of this document, all previous documents become invalid. Invalid originals and copies must be marked as "invalid" by the respective owner, sorted out and replaced by the valid version	

Status	Date	Name/Area	Key	Signature
created	15.11.2022	M. Krommendijk	MK	
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Content **page**

1 Task definition

The aim is to develop a system that can perfuse an isolated porcine liver for 4 to 6 hours. A physiologically relevant environment for the liver should be provided by the system to keep the liver viable and functional during perfusion. The system should also be designed in such a way that perfusion can take place under an MRI scanner.

2 Terms and abbreviations

For terms, abbreviations and symbols not listed here, please refer to the document glossary.

2.1 Terms

Indication

The reason to carry out medical therapy, after assessing the possible risk

Oxygenator

Blood gas exchanger (oxygen and carbon dioxide).

Heat exchanger

Integrated exchanger of heat in oxygenator

Disposable

Item (i.e. tubing, oxygenator or pump head) meant for single use in clinical settings . In this case, items can be re-used when no physical damage is visible.

2.2 Abbreviations

- **O_2**: oxygen
- **CO_2**: carbon dioxide
- **URS:** user requirement specification
- **DRS**: design requirement specification
- **FU:** functional unit
- **N/A:** not applicable

3 Device specifications

The device under development shall include the following functional units:

- FU 1: Liver reservoir
- FU 2: Blood tubing
- FU 3: Arterial pump
- FU 4: Venous pump
- FU 5: Arterial oxygenator
- FU 6: Venous oxygenator

Not part of the development project, but still subject of consideration because of interfaces:

- Arterial pump console (compatible with chosen arterial pump)

- Venous pump console (compatible with chosen venous pump)
- Flow sensors and monitor
- Pressure sensors and monitor
- Heat regulator
- Gas supply
- Additive supply

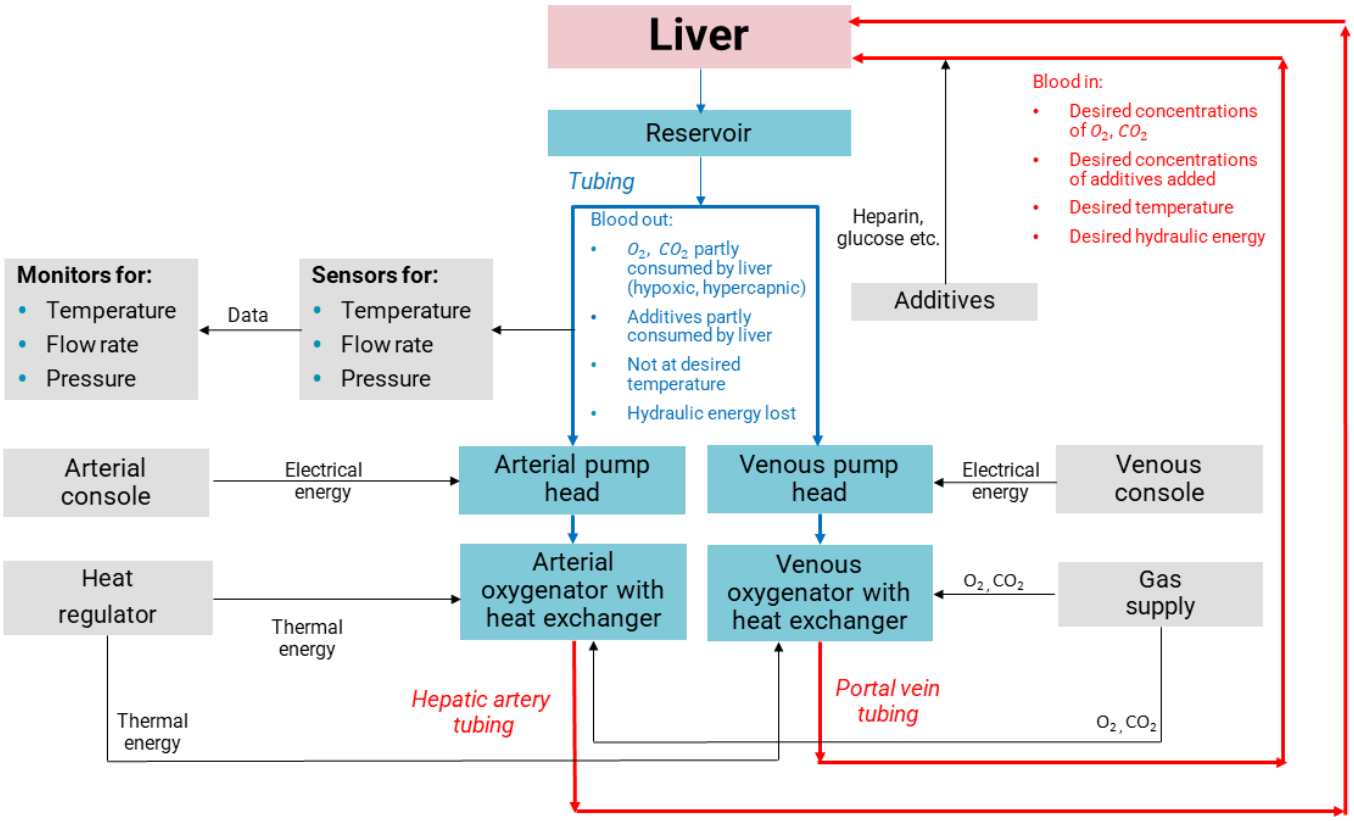

Figure 3-1: System schematic with interfaces of the isolated liver perfusion system

3.1 Intended use, indication

Description of requirement	FU	DRS-ID
The liver reservoir shall be designed to fit a porcine liver	1	3.1-010.000
The liver reservoir shall be made of solid material (plastic) that can bear the weight of the liver (1-3 kg)	1	3.1-020.010
The tubing, pumps, and oxygenators must be compatible with porcine blood	3-6	3.1-030.010
The tubing, pumps, and oxygenators must be compatible with isotonic saline solution	3-6	3.1-030.020

Description of requirement	FU	DRS-ID
The system must be driven from the control room of the MRI while the liver is placed in the reservoir in the MRI room	1	3.1-040.010
The driving system must be separated from the liver in the MRI room by at least 8 meters of tubing	2	3.1-040.020
N/A		3.1-050.000

3.2 Classification and duration of use

Description of requirement	FU	DRS-ID
The product shall be authorised for a period of use up to 8 hours per day (6 hours of perfusion, 2 hours of setup and cleaning).	1-6	3.2-010.010
The system is built for the purpose of research, so no authorisations are required for implantation or other clinical methods	1-6	3.2-010.020
N/A	1-6	3.2-010.030
Since the system will not be used in clinic, and only for research purposes, the system does not need approval as a medical device	1-6	3.2-010.040
N/A		3.2-010.000
N/A		3.2-020.000

3.3 Shelf life

Description of requirements	FU	DRS-ID
The shelf life of the pumps shall be several years	3,4	3.3-010.010
Disposables should be disposed of if there is a risk of damage. Otherwise they should be disposed of after one month of use.	1,2,5,6	3.3-020.020

4 Prerequisites for use

Description of requirements		DRS-ID
The heat regulator used must be able to provide a blood temperature between 35 to 39 °C	Periphery	4.0-010.010
The arterial oxygenator must have an integrated heat exchanger for temperature regulation	5	4.0-010.020

Description of requirements		DRS-ID
The venous oxygenator must have an integrated heat exchanger for temperature regulation	6	4.0-010.020
N/A		4.0-020.000
N/A		4.0-030.000
N/A		4.0-040.000
N/A		4.0-050.000

5 Application and users

5.1 Application process

Description of requirements	FU	DRS-ID
Pump heads must be disassembled easily from the pump drives, with magnetic couplings	3,4	5.1-010.010
Tubing must be able to be disconnected from oxygenators and pump heads easily	2	5.1-020.010
Flow sensors must be either clamp-on or otherwise connected to the tubing	Peri-phery	5.1-020.020
Pressure sensors must be either clamp-on or otherwise connected to the tubing	Peri-phery	5.1-020.030

5.2 Users

Description of requirements	FU	DRS-ID
The intended user is regarded as (or supervised by) an engineering student, medical student or medical professional	_	5.2-010.000
N/A		5.2-020.000
The user must get an introduction on working with porcine blood and organs before operating the system	_	5.2-030.010
N/A		5.2-040.000

6 Device design

Description of requirements	FU	DRS-ID
The system (containing all functional and periphery elements except for the liver reservoir and tubing) shall be placed on a mobile cart/trolley	3-6 and periph-ery	6.0-010.000
The driving system should be placed in the control room of the MRI or on the 0.5mT safety line of the MRI scanner or		6.0-020.000
N/A		6.0-030.000

6.1 Dimensions, Tolerances, Weight

Description of requirements	FU	DRS-ID
The product shall not exceed a spatial dimension of 70 cm in length and width and 200 cm in height.	1-6	6.1-010.010
The weight of the product shall not exceed 100 kg.	1-6	6.1-020.010
Tubing must be disconnected easily for cleaning/disposal	2	6.1-030.010
Pump heads must be disconnected easily for cleaning/disposal	3,4	6.1-030.020
Oxygenators must be disconnected easily for cleaning/disposal	5,6	6.1-030.030

6.2 Materials

Description of requirements	FU	DRS-ID
The liver reservoir shall be made of a hemocompatible plastic	1	6.2-010.010
Tubing shall be made of hemocompatible PVC	2	6.2-010.020
Pump heads shall be made of hemocompatible material	3,4	6.2-010.030
Oxygenators shall be made of hemocompatible material	5,6	6.2-010.040
The entire system shall be cleanable with water and/or saline soluti-on.	1-6 and periph-ery	6.2-020.000
The liver reservoir must be free of any ferromagnetic materials	1	6.2-030.010
The tubing (and tubing connectors) must be free of any ferromagnetic materials		6.2-030.020

Description of requirements	FU	DRS-ID
	2	6.2-030.030
All other materials that do contain metal are to be placed in the control room, not to be placed near the magnet	3-6 and periphery	6.2-030.040
All materials used (especially tubing, pump heads, oxygenators) shall be DEHP free	1-6 and periphery	6.2-040.010
All materials used (especially tubing, pump heads, oxygenators) shall be latex free	1-6 and periphery	6.2-050.010
N/A		6.2-060.000
N/A		6.2-070.000

6.3 Connection techniques

Description of requirements	FU	DRS-ID
All disposables (pump heads, oxygenators, tubing) must be able to disconnect during operation	2-6	6.3-010.000
N/A		6.3-020.000
N/A		6.3-030.000
Pump heads must be connected to pump drives according to the instructions of use of the chosen pumps	3,4	6.3-040.000
Tubing shall be connected to the reservoir using cable glands or other connectors	2	6.3-050.010
Tubing shall be connected to all other elements using connectors corresponding to the respective element (oxygenator, pump head)	2	6.3-050.020

6.4 System design/structure

Description of requirements	FU	DRS-ID
The flow sequence when used as a liver perfusion system should be as follows (for both lines, arterial and venous): • Liver reservoir • 8 meters of tubing (hepatic artery and portal vein) • Pump heads • Tubing and connectors • Oxygenators • 8 meters of tubing • Liver (into cannulas of hepatic artery and portal vein)	1-6	6.4-010.010
N/A		6.4-020.000

6.5 Cleaning/ Reprocessing/Sterilisation/Desinfection

Description of requirements	FU	DRS-ID
The driving system (periphery units) are to be disinfected using ethanol after use	periphery	6.5-010.010
The blood-contact parts of the system are to be cleaned using a specified cleaning solution containing citric acid, pepsin and water	1-6	6.5-020.000
N/A		6.5-030.000

6.6 Packaging

Description of requirements	FU	DRS-ID
Tubing is to be stored in their delivered packaging when not used for long periods of time	2	6.6-010.000
Pump heads are to be stored in their delivered packaging when not used for long periods of time	3,4	6.6-010.020
Oxygenators are to be stored in their delivered packaging when not used for long periods of time	5,6	6.6-010.030
N/A		6.6-020.000
N/A		6.6-030.000

7 Features/Functions

Description of requirements	FU	DRS-ID
Arterial blood flow is to be mimicked using a pulsatile pump	3	7.0-010.010
Venous blood flow is to be mimicked using a continuous flow pump	4	7.0-020.010
Arterial blood is to be oxygenated using one oxygenator	5	7.0-030.010
Venous blood is to be oxygenated using a second oxygenator	6	7.0-030.020
Flow rate and pressure are be monitored during perfusion using flow rate and pressure sensors	Periph-ery	7.0-040.010

7.1 Functional unit 1: Liver reservoir

Description of requirements	FU	DRS-ID
The base of the reservoir must be at least 40x40 cm to fit a porcine liver	1	7.1-010.010
The liver reservoir must not exceed a spatial measureent (in any di-rection) of 60 cm to be able to fit in the bore oft he MRI scanner	1	7.1-020.010
N/A		7.1-030.000

7.2 Functional unit 2: Blood tubing

Description of requirements	FU	DRS-ID
Raumedic blood compatible tubing with an inner diameter of either ¼” or 3/8” shall be used	2	7.1-010.010
N/A	2	7.1-020.010
N/A	2	7.1-030.000

7.3 Functional unit 3: Arterial pump

Description of requirements	FU	DRS-ID
The arterial pump must provide flow rates up to 1000 mL/min	3	7.3-010.010
The pump head must be made from a biocompatible material	3	7.3-020.010
The pulsatile waveform of the blood flow must be controllable using the pump settings	3	7.3-020.020

Description of requirements	FU	DRS-ID
The pump shall be used according to the instructions for use supplied by the manufacturer	3	7.3-030.010

7.4 Functional unit 4: Venous pump

Description of requirements	FU	DRS-ID
The arterial pump should be able to provide flow rates up to 1500 mL/min	4	7.3-010.010
The pump head must be made from a biocompatible material	4	7.3-020.010
The pump shall be used according to the instructions for use supplied by the manufacturer	4	7.3-030.010

7.5 Functional unit 5: Arterial oxygenator

Description of requirements	FU	DRS-ID
The arterial oxygenator must be compatible with gas flows below the arterial blood flow rate (0 to 1000 mL/min)	5	7.3-010.010
The arterial oxygenator must have an integrated heat exchanger for temperature regulation	5	7.3-020.010
The oxygenator shall be used according to the instructions for use supplied by the manufacturer	5	7.3-030.010

7.6 Functional unit 6: Venous oxygenator

Description of requirements	FU	DRS-ID
The venous oxygenator must be compatible with gas flows below the venous blood flow rate (0 to 1500 mL/min)	6	7.3-010.010
The venous oxygenator must have an integrated heat exchanger for temperature regulation	6	7.3-020.010
The oxygenator shall be used according to the instructions for use supplied by the manufacturer	6	7.3-030.010

8 Operation and control, interfaces

8.1 Interfaces system to periphery

Description of requirements	FU	DRS-ID
Pump consoles must have a digital interface for control of pump settings (preferably by use of a display)	Periphery	8.1-010.010
Sensors must have a digital interface to a PC for data transport	Periphery	8.1-020.010
Pump heads must have interfaces for blood flow	Periphery	8.1-030.010
Oxygenators must have an interface for blood flow	4,5	8.1-040.010
Oxygenators must have an interface for gas supply	4,5	8.1-050.010

8.2 Human-system interfaces

Description of requirements	FU	DRS-ID
N/A		8.2-010.000
N/A		8.2-020.000
N/A		8.2-030.010
N/A		8.2-030.020
N/A		8.2-040.000

9 Provision of information by the manufacturer

Description of requirements	FU	DRS-ID
All manuals must be provided in English		9.0-010.010
All labelling must be provided in English		9.0-010.020
N/A		9.0-020.000

9.1 Instructions for Use (IfU)

Description of requirements	FU	DRS-ID
Manuals provided by the manufacturers of the functional units (pumps, oxygenators, sensors and heat regulator) must be taken into account during use		9.1-010.010
N/A		9.1-010.020

9.2 Labeling primary product

Description of requirements	FU	DRS-ID
All non-disposable elements (pumps, sensors, heat regulator) must be labeled with ‘Liver-Twin M3i’	3-4 and periph-ery	9.2-010.010
Tubing must be labeled with „hepatic/arterial“ and „portal/venous“ for consistent use of arterial and venous in-and outflows	2	9.2-020.020
If two of the same oxygenators are used, they must be labeled with „hepatic/arterial“ and „portal/venous“ for consistent use of arterial and venous oxygenators	5,6	9.2-020.030
Oxygenators must have labeled blood in-and outflows (if not already specified by manufacturer)	5,6	9.2-020.040

9.3 Labeling Packaging

Description of requirements	FU	DRS-ID
All packaging of non-disposable elements (pumps, sensors, heat re-gulator) must be labeled with ‘Liver-Twin M3i’	3,4 and periph-ery	9.3-010.010
N/A		9.3-020.000
N/A		9.3-030.000

9.4 Training documents

Description of requirements	FU	DRS-ID
N/A		9.4-010.000

10 Storage and transport conditions

Description of requirements	FU	DRS-ID
The system must be stored at normal room temperature	1-6	10.0-010.010
The system must be transported either inside (within one building) or outside (between buildings) at the specified temperatures of 10°C -30 °C	1-6	10.0-020.010
For temperatures outside of this range, transport time must not exceed 10 minutes to prevent long exposure to sub-optimal temperatures	1-6	10.0-020.020
N/A		10.0-030.020

11 External specifications

Description of requirements	FU	DRS-ID
Disposables must be checked for physical damage regularly when reused more than once	2-6	11.0-010.010
N/A		11.0-020.000

11.1 Economic demands

Description of requirements	FU	DRS-ID
N/A		11.1-010.000
N/A		11.1-020.000

11.2 Legal provisions/standards

Description of requirements	FU	DRS-ID
N/A		11.2-010.000
N/A		11.2-020.000
N/A		11.2-030.000
N/A		11.2-040.000

12 Production and further treatment

Description of requirements		DRS-ID
N/A		12.0-010.000

12.1 Production

Description of requirements		DRS-ID
N/A		12.1-010.000
N/A		12.1-020.000

13 Maintenance/service

Description of requirements		DRS-ID
Maintaince of the arterial pump must occur as specified by the manufacturer	3	13.0-010.010
Maintaince of the venous pump must occur as specified by the manufacturer	4	13.0-010.020
Maintaince of the heat regulator must occur as specified in the manual	periphery	13.0-010.030

14 Disposal

Description of requirements		DRS-ID
N/A		14.0-010.000
N/A		14.0-020.000
All disposable elements (as a whole or dismanteled) must fit in a 30L SZA-WIVA containers for biological waste	1-6	14.0-030.010
Biological waste bins are to be carried off within three days of disposal		14.0-030.020
N/A		14.0-040.000

15 Notes and applicable documents

15.1 Applicable documents

- URS is coupled to corresponding DRS

16 Document revision status

Create date	Revision	Author	Brief description of the change
25.11.2021	00	J. Arens	First version of template
15.11.2022	01	M. Krommendijk	Finished draft for master thesis

17 Attachments

A.4 Additional material equipment choice

Table A5 below presents an overview of the technical specifications of flow sensors by Transonic, Sonotec, and em-tec.

Brand	Flow sensor type	Tubing size (ID)	Max. flow rate (lpm)	Resolution (mL/min)	Max. zero offset (mL/min)	Absolute accuracy	Relative accuracy	Repeatability
Transonic Precision Clamp-On flow sensors ME-PXL Series[64]	2PXL	3/32"	1	0.5	±4.0	±10% (of measured value)	±4% (of measured value)	Not specified
	3PXL	1/8"	2	1	±8.0			
	4PXL	1/8"	2	1	±8.0			
	5PXL	3/16"	2	1	±8.0			
	6PXL	1/4"	5	2.5	±15			
	7PXL	1/4"	10	5	±30			
	8PXL	3/8"	10	5	±30			
	9PXL	3/8"	10	5	±30			
	10PXL	1/2"	20	10	±60			
	11PXL	1/2"	20	10	±60			
	12PXL	1/2"	20	10	±60			
	14PXL	5/8"	50	25	±150			
	14-16PXL	3/4"	50	25	±150			
	20PXL	1"	100	50	±300			
Sonotec Sonoflow CO.56 Ultrasonic Flow Bubble Sensors[65]	CO.56/035	3.0 mm	3	Not specified	±20* in 24h at zero flow	Not specified**	±15 mL/min (5%)	±6 mL/min (2%)
	CO.56/044	3.0 mm	5				±25 mL/min (5%)	±10 mL/min (2%)
	CO.56/060	5.0 mm	6				±30 mL/min (5%)	±12 mL/min (2%)
	CO.56/080	6.0 mm	8				±40 mL/min (5%)	±16 mL/min (2%)
	CO.56/120	10.0 mm	12				±60 mL/min (5%)	±24 mL/min (2%)
	CO.56/140	12.0 mm	14				±70 mL/min (5%)	±28 mL/min (2%)
Em-tec SonoTT Clamp-On Transducers[66]	CT6.8mm	11/64"	±6	Not specified	±30*** in 2h	Not specified**	±70 mL/min****	Not specified
	CT3/16x1/16"	3/16"	±6					
	CT1/4x1/16"	1/4"	±8					
	CT1/4x3/32"	1/4"	±8					
	CT3/8x1/16"	3/8"	±10					
	CT3/8x3/32"	3/8"	±10					
	CT1/2x3/32"	1/2"	±20					

Table A5: table of technical specifications for the Transonic, Sonotec and Em-tec flow sensors. These specifications include sensor type, compatible tubing size, maximum flow rate, resolution, maximum zero offset, absolute and relative accuracy and repeatability.
∗in 24h at 0 flow.
∗∗ Accuracy depends on tubing type and conditions such as temperature and fluid properties. Absolute accuracy is determined by zero stability, resolution and zero offset effects.
∗ ∗ ∗ in 2h.
∗ ∗ ∗∗ 70mL/min (+offset) holds for flows below 1 lpm. For higher flow rates, the accuracy is ±7% the measured value + offset.

The Honeywell pressure sensor (40PC015G transducer by Honeywell Inc., Freeport, Illinois, USA) is used for pressure measurements. This sensor has the following specifications:

- Pressure range: 0-15 psi (0-776 mmHg)
- Output voltage: 0-5 V
- Total accuracy: 4.0 %
- Sensitivity: 266.6 mV/psi

A.5 Reference values for hemodynamic parameters from literature

Table A6 below presents the reference values for the hemodynamic parameters found in literature. In this table, clinical values for flow rate and pressure in the hepatic artery, portal vein and hepatic vein are presented. They are based on imaging experiments on healthy patients or donor livers or they are based on perfusion models from CT images.

Source	Vessel	Subjects	Measurement method	Flow rate (mL/min)	Value/range	Pressure (mmHg)	Value/range
Basciano et al.[67]	Hepatic artery (common)	Waveform extrapolated from data by Carlisle et al.[68]	Doppler US	250-850	Range from waveform	75-170	Range from waveform
Bombelli et al.[69]	Hepatic artery	22 healthy subjects, 11 male	Doppler US	350 male 270 female	Mean value		Mean value
Carlisle et al.[68]	Hepatic artery (common)	10 healthy subjects, female	Doppler US	359	Mean value	_	_
Carlisle et al.[68]	Hepatic artery (proper)	10 healthy subjects, female	Doppler US	212	Mean value	_	_
Debbaut et al.[70]	Hepatic artery	2 healthy donor livers	Electrical liver perfusion model from CT images	350	Mean value	100	Mean value
Ma et al.[43]	Hepatic artery (proper)	1 healthy subject	Hepatic perfusion model from CT images	60-900	Range from waveform	75-120	Range from waveform
Săftoiu et al.[71]	Hepatic artery (proper)	9 healthy subjects	Doppler US	224	Mean value	_	_
Yzet et al.[72]	Hepatic artery (proper)	9 healthy subjects	PC-MRI	215	Mean value	_	_
Yzet et al.[72]	Hepatic artery (proper)	9 healthy subjects	Doppler US	306	Mean value	_	_
Bombelli et al.[69]	Portal vein	22 healthy subjects	Doppler US	1230 male 800 female	Mean value		Mean value
Carlisle et al.[68]	Portal vein	10 healthy subjects, female	Doppler US	724.2	Mean value	_	_
Debbaut et al.[70]	Portal vein	2 healthy donor livers	Electrical liver perfusion model from CT images	1100	Mean value	10	Mean value
Ma et al.[43]	Portal vein	1 healthy subject	Hepatic perfusion model from CT images	570-700	Range from waveform	8-9	Range from waveform
Marambio et al.[73]	Portal vein	291 liver transplants	Transit time flow US	123/100g liver weight	Mean value		Mean value
Săftoiu et al.[71]	Portal vein	9 healthy subjects	Doppler US	1322	Mean value	_	_
Yzet et al.[72]	Portal vein	9 healthy subjects	PC-MRI	986	Mean value	_	_
Yzet et al.[72]	Portal vein	9 healthy subjects	Doppler US	993	Mean value	_	_
Debbaut et al.[70]	Hepatic vein	2 healthy donor livers	Electrical liver perfusion model from CT images	1450	Mean value	3	Mean value
Ma et al.[43]	Hepatic vein	1 healthy subject	Hepatic perfusion model from CT images	705-977	Range from waveform	5.38-7.20	Range from waveform

Table A6: An overview presenting flow rate and pressure values found in literature for the hepatic artery, portal vein and hepatic vein.

A.6 Additional results of flow and pressure measurements

A.6.1 Flow and pressure measurements before and after oxygenators

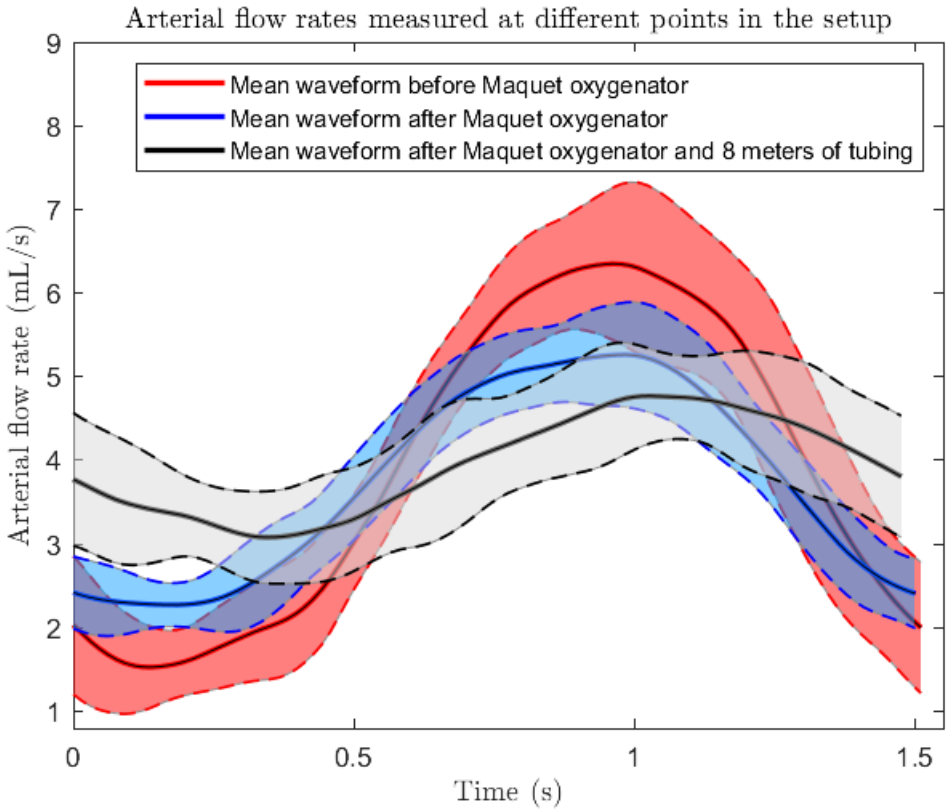

Figure A1: Mean arterial flow waveforms measured at different points in the setup. Of each measurement of flow rate over time, 10 periods were isolated and a mean waveform was calculated and plotted in this graph. The red, blue and black graphs show measurements before the Maquet oxygenator, after the Maquet oxygenator and after 8 meters of tubing respectively.

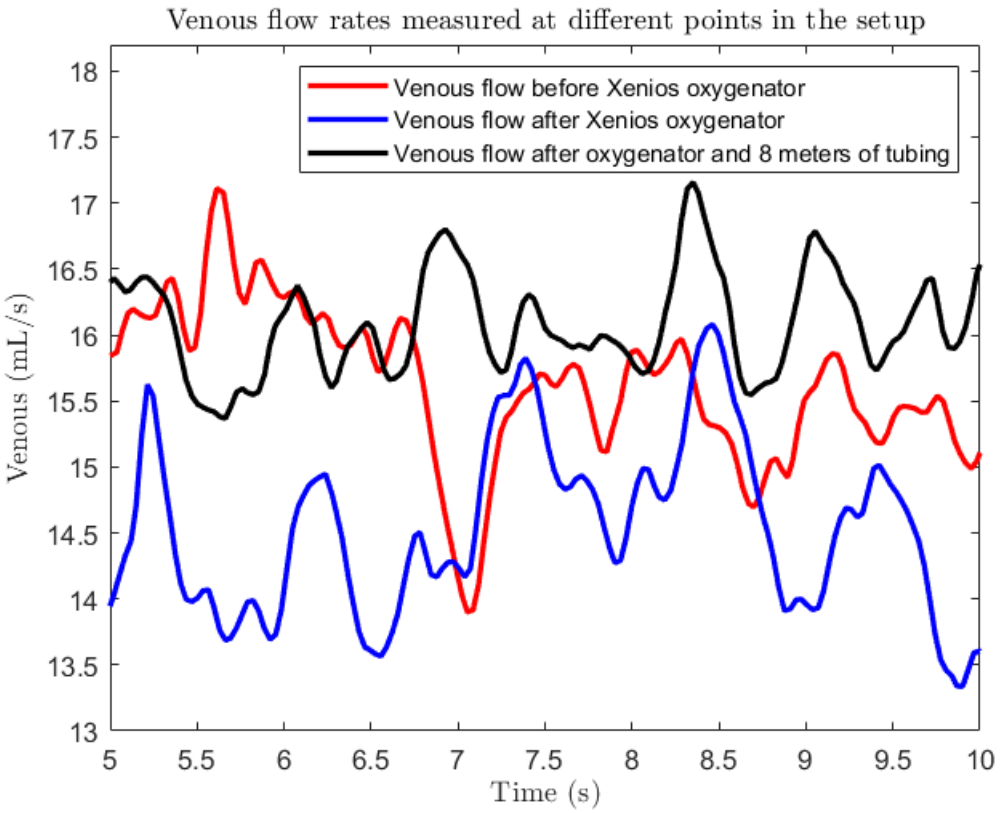

Figure A2: Venous flow rates measured at different points in the setup. The red, blue and black graphs show measurements before the Maquet oxygenator, after the Maquet oxygenator and after 8 meters of tubing respectively.

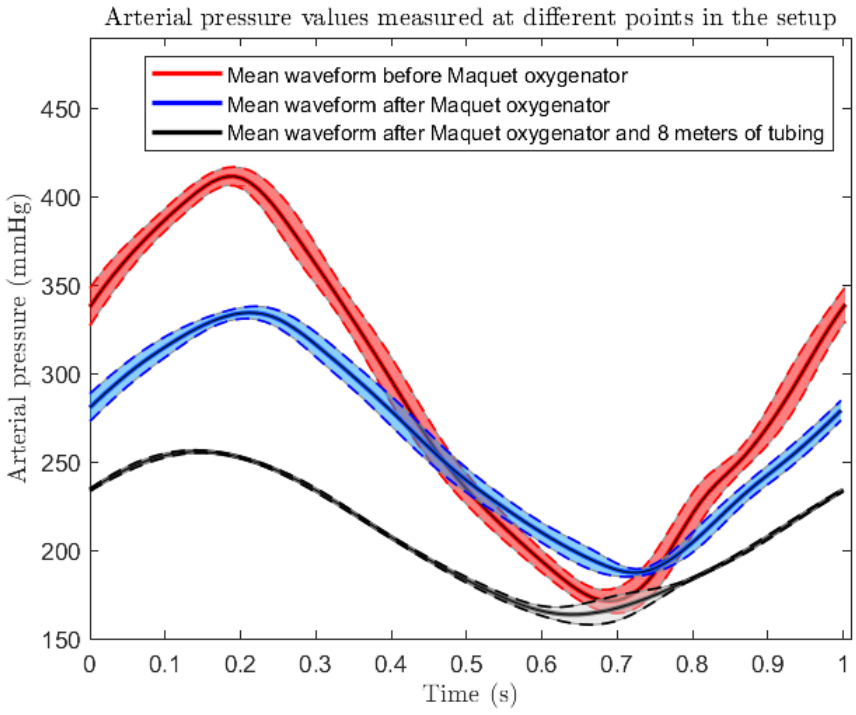

Figure A3: Mean arterial pressure waveforms measured at different points in the setup. Of each measurement of pressure over time, 10 periods were isolated and a mean waveform was calculated and plotted in this graph.The red, blue and black graphs show measurements before the Maquet oxygenator, after the Maquet oxygenator and after 8 meters of tubing respectively.

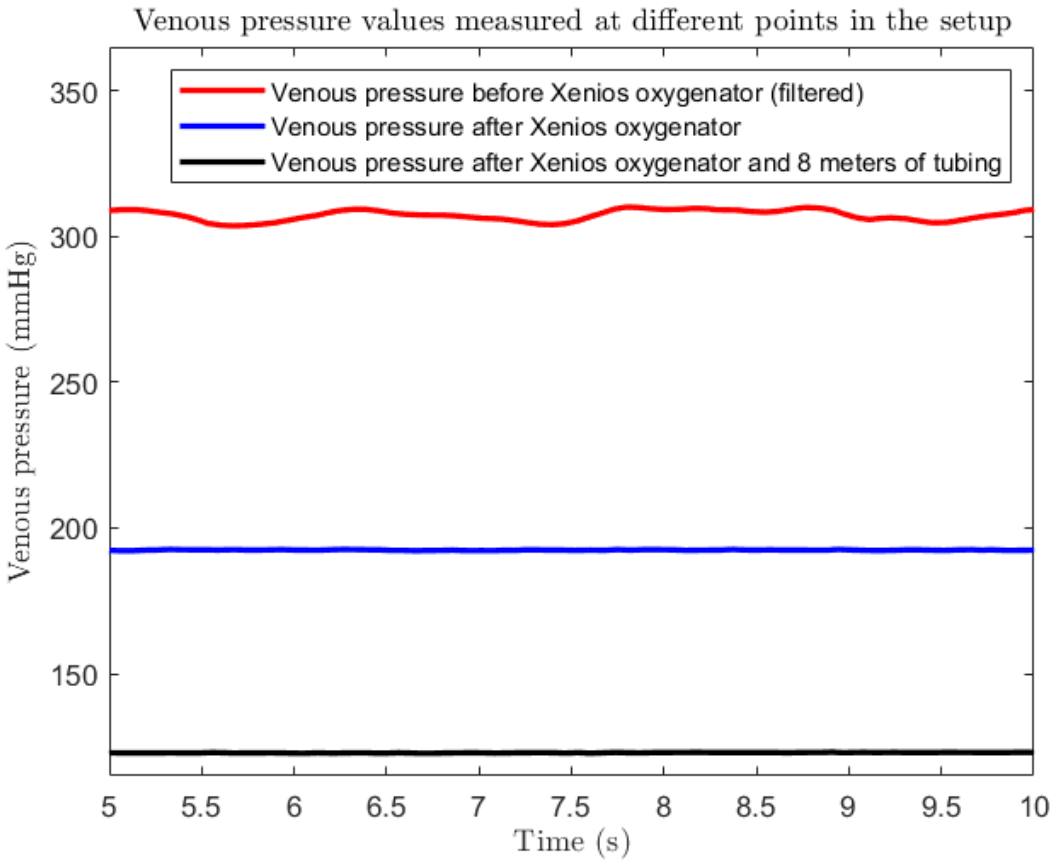

Figure A4: Venous pressure values measured at different points in the setup. The red, blue and black graphs show measurements before the Maquet oxygenator, after the Maquet oxygenator and after 8 meters of tubing respectively.

A.6.2 Calibration curve Sonotec flow sensor

Figure A5 below presents the calibration curve for the Sonotec (Sonoflow CO.55/060 V2.0) flow sensor. This curve was made by applying continuous flow using the Maquet Rotaflow pump (0-3000 rpm), clamping the sensor onto the 1/8" blood-compatible tubing. The applied flow rate was measured using a beaker and stop-watch (plotted on the x-axis) and measuring the resulting flow using (plotted on the y-axis). Mean values and standard deviation errorbars are plotted in this graph, through which a linear fit was calculated using MATLAB's curve-fitting tool.

Since this curve was performed with water, and not blood, it was not used to calibrate the measured data presented in this thesis. It is recommended to do a proper calibration procedure using blood and blood-compatible tubing for future experiments (see discussion).

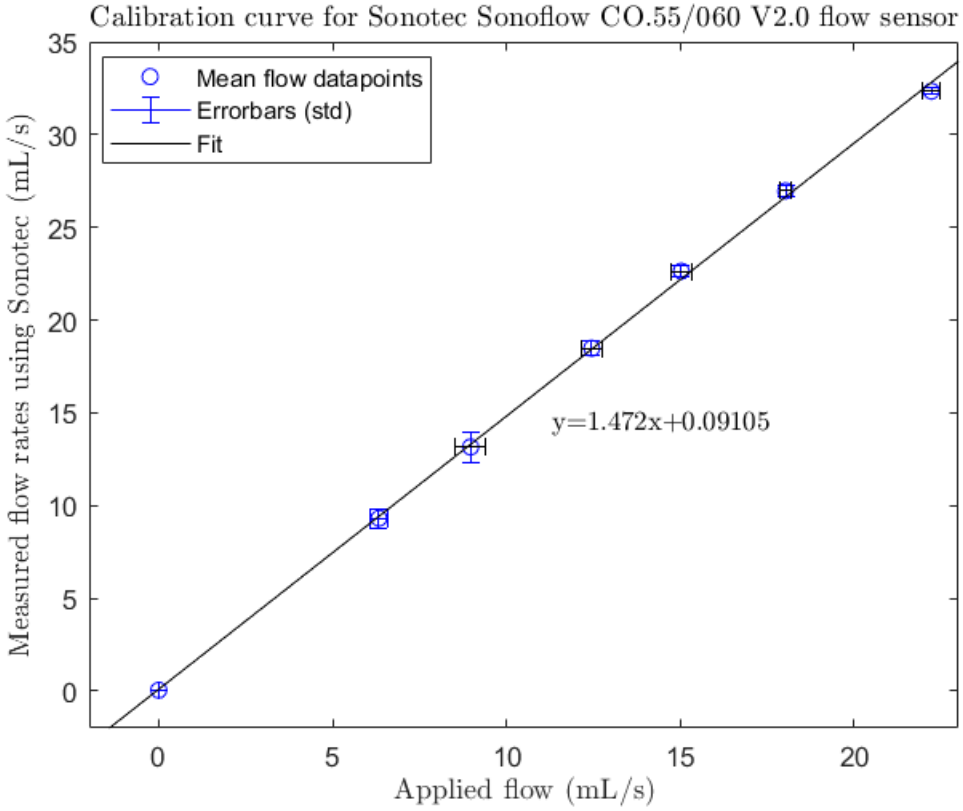

Figure A5: Calibration curve for the Sonoflow flow sensor (CO.55/060 V2.0) by Sonotec.

A.6.3 Calibration curve Honeywell pressure sensor

Figure A6 below presents the calibration curve for the 40PC015G pressure transducer by Honeywell. A manometer was used to apply pressure values between 0 and 300 mmHg and pressure was measured using one of the transducers connected to the tip of the manometer. The measured voltages for each measurement were saved, mean values were taken and plotted against the applied pressure. Using MATLAB's curve-fitting tool, a linear fit was drawn through these datapoints. This fit was used to convert the measured voltages to pressure values in mmHg.

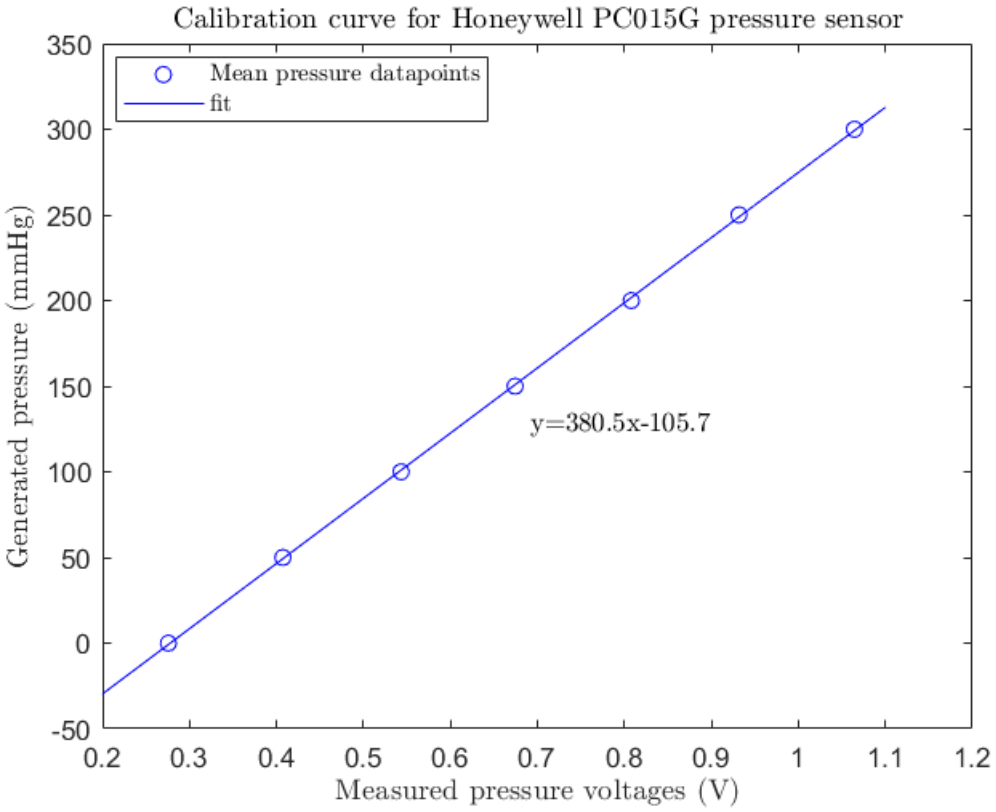

Figure A6: Calibration curve for the pressure transducer (40PC015G) by Honeywell.

A.6.4 Arterial flow waveform for saline solution

Figure A3 below presents the mean arterial flow waveform before the Maquet oxygenator with saline solution. The following pump settings were applied during this measurement:

Xenios pump:

- $rpm = 5600$
- $\Delta rpm = 1400$
- $pulsefrequency = 60Hz$
- $systole = 50\%$

Maquet pump:

- $rpm = 3580$

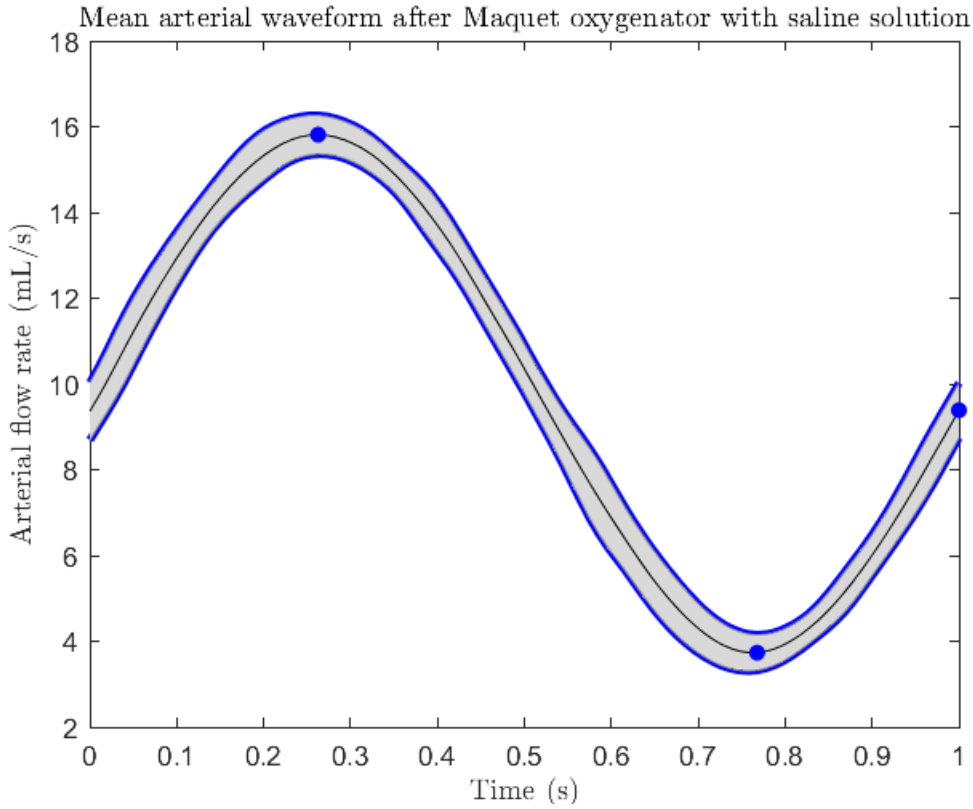

Figure A7: Mean arterial flow waveform before Maquet oxygenator with saline solution.

A.7 Settings MRI experiments

Scanner: Siemens MAGNETOM Aera 1.5T scanner (Siemens, Munich, Germany)
Contrast agent: Dotarem (Gadoteric acid - Gadoterate meglumine by Guerbet, Villepinte, France) at a concentration of 0.5 mmol/mL

Saline solution perfusion

T1-weighted anatomical scans (pre-and post-gadolinium):

- Sequence: $*se2d1$ (spin echo)
- Field-of-view FOV = 290 mm x 320 mm
- Repetition time TR = 528.0 ms
- Echo time: TE= 13.0 ms
- Flip angle = 90.0 degrees

T2-weighted anatomical scan:

- Sequence: $tse2d1\_17$ (turbo spin echo)
- Field-of-view FOV = 290 mm x 320 mm
- Repetition time TR = 5000.0 ms
- Echo time: TE= 96.0 ms
- Flip angle = 150.0 degrees

Angiography scan:

- Sequence: $fl3d1$ (FLASH, fast low angle shot)
- Field-of-view FOV = 312 mm x 384 mm
- Repetition time TR = 3.01 ms
- Echo time: TE= 1.04 ms
- Flip angle = 22.0 degrees

Dynamic T2-weighted scan:*

- Sequence: $epfid2d1\_128$ (echo-planar imaging)
- Field-of-view FOV = 128 mm x 128 mm
- Repetition time TR = 1600.0 ms
- Echo time: TE= 30.0 ms
- Flip angle = 90.0 degrees

Blood perfusion

T1-weighted anatomical scan:

- Sequence: $se2d1$ (spin echo)
- Field-of-view FOV = 312 mm x 384 mm
- Repetition time TR = 528.0 ms
- Echo time: TE= 13.0 ms
- Flip angle = 90.0 degrees

T2-weighted anatomical scan:

- Sequence: $tse2d1\_17$ (turbo spin echo)
- Field-of-view FOV = 348 mm x 384 mm
- Repetition time TR = 5000.0 ms
- Echo time: TE= 97.0 ms
- Flip angle = 150.0 degrees

Angiography scan:

- Sequence: $fl3d1$ (FLASH, fast low angle shot)
- Field-of-view FOV = 312 x 384 mm
- Repetition time TR = 2.21 ms
- Echo time: TE= 0.86 ms
- Flip angle = 19.0 degrees

Dynamic T2-weighted scan:*

- Sequence: $epfid2d1\_128$
- Field-of-view FOV = 128 mm x 128 mm
- Repetition time TR = 1600.0 ms
- Echo time: TE= 30.0 ms
- Flip angle = 90.0 degrees

A.8 Derivation formula for perfusion quantification

This section describes the derivation of equation 1. Adapted from derivations found in literature.[74–76]

Starting off with defining $\Delta R2^*$ as the change in observed relaxation rate (the inverse of $T2^*$ relaxation time). This parameter is directly related to the concentration of gadolinium-based contrast agent $[Gd]$. We can define the following two equations for these statements:

$$\Delta R_2^* = 1/T2^* \tag{2}$$

$$\Delta R_2^* \propto [Gd] \tag{3}$$

Now, define the signal generated by the simple gradient echo sequence as S. This parameter has the following exponential relation to relaxation time $T2^*$ and echo time TE:

$$S = ke^{-TE/T2^*} \tag{4}$$

The parameter k is an unknown proportionality factor — dependent on tissue properties, contrast agent type, and measurement sequence — and is assumed to be equal for liver tissue and large vessels.[21]

Substitution of equation 2 can be performed and after taking the natural logarithm, this equation can be simplified.

$$S = ke^{-TER2^*} \tag{5}$$

$$\ln(S/k) = \ln e^{-TER2^*} \tag{6}$$

$$\ln(S/k) = -TER2^* \tag{7}$$

$$R2^* = -\frac{1}{TE}\ln(S/k) \tag{8}$$

Now, let us define $R2_0^*$ as the relaxation rate before the administration of contrast agent and $R2_t^*$ as the relaxation at timepoint t while contrast agent is being administered:

$$R2_0^* = -\frac{1}{TE}\ln\left(\frac{S_0}{k}\right) \tag{9}$$

$$R2_t^* = -\frac{1}{TE}\ln\left(\frac{S_t}{k}\right) \tag{10}$$

The change in relaxation rate can be defined as the difference between the relaxation rate at timepoint t and the relaxation rate before contrast administration:

$$\Delta R2^* = R2_t^* - R2_0^* \tag{11}$$

Combing this relation with equation 8 results in the following equation for the change in relaxation rate related to signal intensity:

$$\Delta R2^* = -\frac{1}{TE}\left(\ln\left(\frac{S_t}{k}\right) - \ln\left(\frac{S_0}{k}\right)\right) \tag{12}$$

$$\Delta R2^* = -\frac{1}{TE}\ln\left(\frac{S_t}{S_0}\right) \tag{13}$$

We can now substitute equation 3 into this equation to give the approximated contrast agent concentration. Echo time can be ignored since it is constant over all scans — and factor k is uknown and disregarded — so we consider this equation to give the 'scaled tissue perfusion factor' or $STPF$ for short. When tracked over time, this parameter is related to the signal intensity over time $S(t)$.

$$STPF(t) \propto [Gd](t) = -\ln\left(\frac{S(t)}{S_0}\right) \tag{14}$$

A.9 Additional results MRI experiments

Figures A8 and A9 show the T1-weighted and T2-weighted anatomical scans of the liver perfused with blood. On these scans, artefacts can be observed near the edges of the liver (indicated by the red arrows).

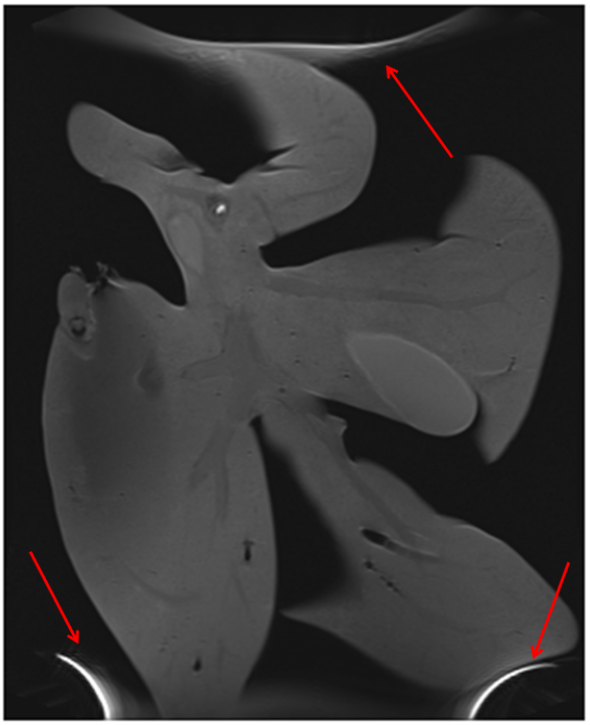

Figure A8: A single slice of the T1-weighted scan of the liver perfused with blood. The scan was performed using a 2D spin echo sequence.

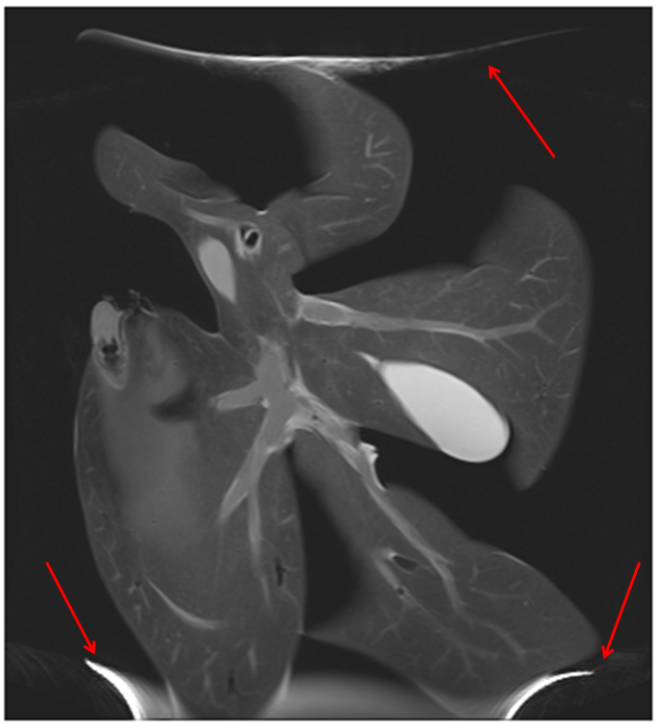

Figure A9: A single slice of the T2-weighted scan of the liver perfused with blood. The scan was performed using a 2D turbo spin echo sequence.

These artefacts are caused by bending of magnetic field lines near the edges of the reservoir (see figure A10). These are visible in the scan since a large FOV had to be chosen to fit the entire liver in the frame.

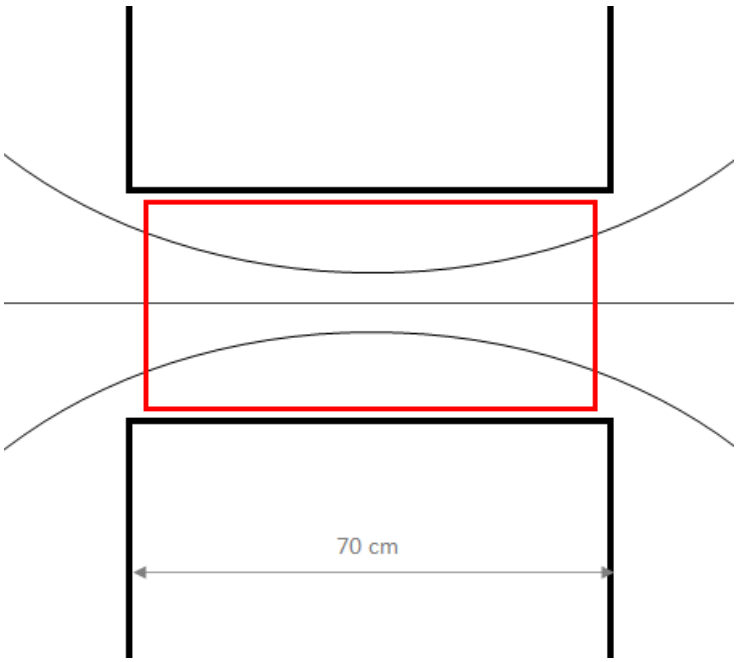

Figure A10: Side-view of the reservoir in the MRI. The magnet tunnel is relatively small (70 cm). The magnetic field lines bend at the edges of the tunnel. As a large field-of-view is chosen, these bent field lines cause artefacts at the edges of the reservoir.