On-pump vascular reperfusion of Thiel embalmed cadavers

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For Caroline

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List of abbreviations

CCE Cholecystectomy

CENTRAL Cochrane Central Register of Controlled Trials

CT Computed Tomography

DiI 1, 1'-dioctadecyl-3, 3, 3', 3'-tetramethylindocarbocyanine perchlorate

ICG Indocyanine Green

MB Methylene Blue

mmHg Millimeter of Mercury

mPa.s Millipascal.Second (SI Unit of viscosity)

MPMCTA Multiphase Postmortem Computed Tomography Angiography

n.a. Not Applicable

n.r. Not reported

OR Operating Room

OSATS Objective Structured Assessment of Technical Skills

PEG Polyethylene Glycol

PGY Postgraduate Year

PL Paraffinum Liquidum

PMCT Postmortem Computed Tomography

PP Paraffinum Perliquidum

SD Standard Deviation

TAVI Transcatheter Aortic Valve Implantation

TEP Total Extraperitoneal

VR Virtual Reality

VT Video Trainer

Chapter 1

Summary/Samenvatting

Traditionally, surgery has been taught and practiced on real patients in the operating room (OR), which is often time-consuming, may lead to more patient complications and poor patient outcomes. More than a decade ago, the Institute of Medicine increased the public and institutional awareness about the high prevalence of medical errors in modern healthcare.[1] Besides, opportunities for surgical teaching have significantly diminished since the introduction of the European Working Time Directive.[2] As a consequence, educational bodies began to consider methods of training that would improve physician education without putting the patient at risk by using a wide range of training models. Within this area, soft-embalmed human bodies like Thiel cadavers are notable and suited to train surgical procedures. However, flow from artery to vein in embalmed human bodies to simulate closely the living has not yet been installed. The purpose of this thesis is to establish and analyze a long dynamic flow from artery to vein in Thiel embalmed cadavers.

The first aim of this thesis was to compare various surgical training models, including human cadavers used to learn laparoscopic skills **(chapter 4)**. Training with a virtual reality (VR) simulator or a video trainer (VT) versus no activity improves basic laparoscopic skills. Moreover, the effectiveness of VR and VT to learn minimally invasive surgical skills is similar. Structured stepwise proficiency-based training using validated VR models leads to higher quality performances compared to standard laparoscopic training in the OR. Only one trial, focusing on laparoscopic sigmoidectomy, compared human cadavers versus VR simulators and observed better results for one out of four scores after cadaveric training. Since only four out of 58 trials were free of bias, no further conclusions can be drawn from this review.

During the last decades, researchers have reperfused human cadaver models and used them in surgical training. In **chapter 5**, the literature about the use of these reperfused human cadaver surgical training models was systematically reviewed. Embalmed and fresh tissues have been equally employed. Within the extent of embalmed bodies, a minority of studies reported training on Thiel embalmed models. The vessels of complete bodies were filled in half of the reports, while reperfusion of both arteries and veins was carried out in more than one-third of studies. Generally, water-based perfusates were applied, which quickly cause edema and tissue deformation. Furthermore, a prolonged dynamic reperfusion of the entire vascular tree of Thiel embalmed human bodies with a more suitable perfusate than water has not been described. The reported reperfused models were mainly utilized to teach vascular procedures, but training progress was not monitored with validated assessment tools. In two studies, new flap techniques were practised on cadavers and repeated successfully in patients. Regular workshops using reperfused human cadavers currently do not exist.

As a first experimental step, a dynamic flow from the external iliac artery to the external iliac vein was created in one fresh pig hindquarter **(chapter 6)**. A heart-lung machine pumped Paraffinum Perliquidum (PP) through a cannula that was inserted in the external iliac artery. Although venous drainage was realized, the reperfusion was prematurely terminated due to an uncontrollable leakage at the cutting edges of the raw muscle surface and the hemisectioned sacrum. Afterwards, PP drained from an incision made in the skin and subcutaneous tissue of the ankle. Thus, this pilot study demonstrated that PP runs from artery to vein under acceptable conditions, but with significant leakage.

Chapter 7 analyzed if a similar reperfusion can be established in the vessels of one pair of fresh pig lungs. In addition, the pulmonary distribution of PP was evaluated. As described above, a heart-lung machine injected PP into the arterial system (*i.e.* pulmonary trunk). After spread in both lungs, PP eventually left the specimen through a cannula inserted in the left atrium. Imaging showed a diffuse distribution of contrast-enhanced PP in the vascular system of both lungs, which decreased in cranial direction. A significant leakage toward the tracheobronchial tree was measured, most probably due to too high inlet pressures, causing vessel wall rupture. Overall, PP enabled vascular reperfusion in fresh lungs from artery to vein with adequate pressure-flow relationship. However, leakage remained an important issue, necessitating further exploration of the reperfusion capacities of PP.

Next, Thiel embalmed pig kidneys were reperfused **(chapter 8).** Because the quality of tissue preservation in Thiel embalmed human bodies is also known to vary, the spread of the embalming solution in the pig kidney was investigated. In 15 kidneys, contrast-enhanced Thiel embalming solution was administered. Adequate preservation was achieved due to complete parenchymatous dissemination of the solution. Then, we reperfused 20 Thiel embalmed pig kidneys for 120 minutes at a flow rate of 1 mL/min with either PP or diluted Polyethylene Glycol (PEG) 400. No statistically significant weight, volume and pressure gain were observed after more than 60 minutes of reperfusion with PP. In contrast, these parameters further increased during reperfusion with diluted PEG. PP filled both major vessels and renal tissue, whereas diluted PEG spread widely in the kidney. In conclusion, PP circulated in Thiel embalmed pig kidneys, which were suitable to establish this.

The purpose of the next study was threefold **(chapter 9)**. First, establishing and assessing ideal Thiel embalming circumstances. Therefore, immediately after euthanasia, pressure-controlled pump-driven embalming of one complete pig and three pig hemibodies was performed, enabling adequate tissue perfusion and fast administration of the embalming solution. Direct inspection during embalming revealed that arterial filling was followed by dispersion in the

organs and finally venous drainage. After two weeks, further distribution of the embalming solution was noticed, indicating that the process continued.

Subsequently, a long reperfusion from artery to vein with PP was assessed and its effect on the gross anatomy and its feasibility for surgical training were observed. We found that arterially injected PP drained via the venous cannula. Leakage, however, persisted despite a maximum arterial pressure of 30 mmHg, forcing to lower the flow rate once venous drainage occurred. Most organs and tissues, however, tended to have an adequate vascular filling. Subsequent reperfusion for several hours allowed testing several surgical procedures under realistic circumstances.

Next, the microvascular flow of PP in pump-embalmed pig small intestines was investigated and compared with that perceived in gravity-embalmed human tissues. PP recruited the capillaries of pump-embalmed pig small intestines without extravasation and finally filled the veins. In contrast, in gravity-embalmed human small intestines, PP was blocked at the arterial level probably due to multiple brownish spots fixed in the mucosa. The composition of these spots is still unknown. In summary, pressure-controlled fast administration of Thiel embalming solution immediately after death was essential to facilitate vascular reperfusion. The amount of leakage during reperfusion with PP varied between organs, but prolonged reperfusion at low flow rates was possible without major gross anatomical deformations, enabling surgical training.

Chapter 10 mainly presents preliminary results of reperfusion of kidneys, prelevated from Thiel embalmed human cadavers. The same technique was applied as described in chapter 8, but reperfusion was established with PP for 240 minutes. Statistically significant weight and volume gain were observed during the first 120 minutes. Afterwards, no further weight and volume increases were measured. The macrovascular course of PP was nicely visualized with Computed Tomography (CT), but minor accumulations of contrast agent were observed in the cortex, impeding further interpretation of the course of PP. Afterwards, to closely mimic a dynamic lifelike reperfusion, the flow rate was increased to 25 mL/min for one hour, which resulted in statistically significant weight and volume gain despite low arterial pressures.
Similarly, one liver was reperfused for 240 minutes at low flow rates and low vascular pressure. Although a flow from the hilar vessels to the inferior vena cava was effectively installed without obvious gross anatomical deformations, the vascular anatomy could not be clearly reproduced due to widespread minor parenchymatous accumulations of contrast agent.
A flow from artery to vein was also installed for several hours in one human arm. Only minor subcutaneous accumulations of contrast agent were observed after 240 minutes. Moreover, the course of major arteries and veins could be traced up to the level of the hand, but no contrast

was found in the vessels of the fingers. Afterwards, vascular surgery could be practiced on this model under realistic conditions.
Finally, a long reperfusion in a complete Thiel cadaver was intended. Although the vessels of several organs and tissues were realistically filled, only local venous return was observed and no venous outflow occurred in the cannula. Local differences in capillary leakage and presence of arteriovenous shunts; microvascular obstructions; and clots in major veins may be causative factors for lack of venous drainage, hindering a successful dynamic reperfusion in a complete Thiel human cadaver.

Chapter 11 describes how transfemoral aortic valves were implanted in eight Thiel embalmed human bodies. A Virtangio perfusion device injected Paraffinum Liquidum (PL) in the femoral artery in retrograde direction. Due to the expansion of the aorta the introduction and deployment of aortic valves was feasible under fluoroscopic guidance. Five valves were correctly positioned. This reperfusion model may allow training of endovascular transfemoral techniques.

In conclusion, (non)-reperfused human bodies' significance within the extent of surgical training tools is still unclear. PP reproduces a lifelike flow in pump-driven pressure-controlled Thiel embalmed pig models for a prolonged period under acceptable intravascular circumstances, but leakage that varies among organs exists. On-pump embalming is a requisite to ease vascular reperfusion. This reperfusion, however, causes no major gross anatomical deformations, allowing lifelike simulation of multiple surgical operations.
PP runs under allowable intravascular conditions and has tolerable tissue effects in gravity-embalmed Thiel human kidneys, one liver and one arm, but its microscopic course in human tissues (except small intestines) has not yet been demonstrated. Reperfusion of a Thiel embalmed human body is still in a preliminary stage, preventing us to draw robust conclusions. Future anatomical research will focus on Thiel embalming solution's intracorporeal spread during on-pump cadaveric embalming; vascular wash-out techniques prior to embalming; and implementation of pressure-controlled arterial embalming. In addition, the course of several types of perfusates in Thiel embalmed human body parts will be compared. The findings of these experiments can aid in the elaboration of vascular reperfusion in Thiel embalmed human bodies using a preferred perfusate. Finally, various surgical procedures on reperfused Thiel embalmed human organs and extremities can be initiated and assessed.

Van oudsher worden chirurgische vaardigheden aangeleerd op patiënten in de operatiezaal. Dit is tijdrovend en kan leiden tot meer complicaties. In 1999 wees het Amerikaanse instituut voor geneeskunde voor het eerst op de hoge prevalentie van medische fouten in de moderne gezondheidszorg.[1] De invoering van de Europese richtlijn betreffende de organisatie van de arbeidstijd in 2003 zorgde echter voor een belangrijke reductie in operatietijd en dus leermogelijkheden voor chirurgen in opleiding.[2] Om de kunde van chirurgen in opleiding te verbeteren alvorens ze op patiënten opereren wordt in toenemende mate gebruik gemaakt van trainingsmodellen. Er is een uitgebreid aanbod aan trainingsmodellen. Thiels gebalsemde menselijke kadavers zijn uniek om gevorderde chirurgische procedures te oefenen. In Thiels gebalsemde menselijke kadavers werd echter nog nooit een doorbloeding van slagader tot ader aangelegd. Dit doorbloede of gereperfundeerde model kan zo de eigenlijke patiënt nog beter simuleren. Het doel van deze thesis is het installeren en analyseren van een langdurige dynamische doorbloeding van slagader tot ader in Thiels gebalsemde kadavers.

Hoofdstuk 4 van deze thesis vergeleek chirurgische trainingsmodellen om laparoscopische vaardigheden aan te leren. Simulaties met virtuele realiteit (VR) en video trainers (VT) verbeteren in gelijke mate chirurgische basisvaardigheden in vergelijking met geen training. Gestructureerde training met VR leidt tot een betere laparoscopische handigheid dan de traditionele leermethode in de operatiezaal. Slechts één studie vergeleek training op menselijke kadavers met VR. In deze studie werd een sigmoidresectie tijdens een kijkoperatie geoefend. Slechts één op vier geëvalueerde scores was beter na training op menselijke kadavers. De methodologie van deze studie was echter niet goed waardoor de resultaten niet betrouwbaar zijn. Andere onderbouwde besluiten kunnen niet uit deze review genomen worden gezien slechts vier van de 58 studies methodologisch correct uitgevoerd werden.

De voorbije jaren trachtten onderzoekers de bloedvaten van menselijk kadaverweefsel te vullen. Deze gereperfundeerde modellen werden vervolgens gebruikt voor chirurgische training. **Hoofdstuk 5** is een overzicht van menselijke kadavers met gereperfundeerde bloedvaten die gebruikt worden voor chirurgische training. Er wordt even vaak getraind op gebalsemde en verse gereperfundeerde menselijk kadavers. Training op gereperfundeerd Thiels gebalsemd weefsel gebeurt slechts zelden. Bijna de helft van de studies onderzochten reperfusie in volledige kadavers. Bovendien werden slagaders én aders in het merendeel van de studies opnieuw doorbloed. Doorgaans werden waterige perfusievloeistoffen geïnjecteerd die vlot oedeem en weefselvervorming veroorzaken. Er werd echter nog nooit een langdurige dynamische reperfusie van slagader naar ader gerapporteerd. Gereperfundeerde trainingsmodellen lieten toe om talrijke chirurgische procedures te oefenen. Het merendeel betrof vaatchirurgie. Er is momenteel geen enkele studie die de progressie van aangeleerde vaardigheden op gereperfundeerd kadaverweefsel met een gevalideerde methode objectief

nagaat. Slechts in twee studies werden aangeleerde vaardigheden op gereperfundeerde kadavers met succes herhaald in de patiënt. Workshops op gereperfundeerde modellen worden nu nog niet systematisch georganiseerd.

Hoofdstuk 6 beschrijft een dynamische reperfusie van de arteria iliaca externa naar de vena iliaca externa in het achterkwartier van een pas overleden varken. Een hart-longmachine pompte Paraffinum Perliquidum (PP) in een canule die in de arteria iliaca externa was geplaatst. Uiteindelijk draineerde PP langs de vena iliaca externa. Het experiment werd vroegtijdig beëindigd omwille van een lekkage ter hoogte van de doorgesneden spieren en het sacrum. Na het experiment vloeide PP uit een incisie die gemaakt werd ter hoogte van de huid en onderhuids vet van de enkel. Deze pilootstudie toonde aan dat een dynamische reperfusie kan aangelegd worden van slagader tot ader in vers varkensweefsel onder aanvaardbare condities maar met een belangrijke lekkage.

Hierna werd onderzocht of een gelijkaardige reperfusie kan aangelegd worden in de bloedvaten van een paar verse varkenslongen **(hoofdstuk 7)**. Bovendien werd de verspreiding van PP in de longen nagegaan. De hart-longmachine pompte eerst PP in de truncus pulmonalis. PP verspreidde zich vervolgens in de longen en draineerde tenslotte langs een canule in de linker voorkamer. Beeldvorming toonde dat PP met contrast zich overal in de longen bevond maar met afnemende mate in craniale richting. We maten een belangrijke lekkage naar de luchtwegen. Wellicht veroorzaakten te hoge intravasculaire drukken een ruptuur van de bloedvatwand. We concludeerden dat PP van slagader tot ader circuleert in een paar verse varkenslongen met aanvaardbare intravasculaire druk en debiet. Lekkage bleef echter een probleem waardoor de reperfusie-eigenschappen van PP verder moeten worden geanalyseerd.

In een volgend experiment **(hoofdstuk 8)** reperfundeerden we Thiels gebalsemde varkensnieren. We onderzochten ook de verspreiding van de balsemvloeistof aangezien de balsemkwaliteit van menselijke kadavers varieert. Hiervoor balsemden we 15 nieren met een mengsel van contraststof en Thielse balsemvloeistof. Een volledige verspreiding van de balsemvloeistof in het nierparenchym zorgde ervoor dat alle gebalsemde nieren intact bewaard werden. Hierna reperfundeerden we 20 Thiels gebalsemde varkensnieren gedurende 120 minuten aan 1 mL/min met PP of verdunde Polyethyleenglycol (PEG) 400. Na meer dan 60 minuten reperfusie met PP stelden we geen significante gewichts-, volume- en intravasculaire druk toename vast. Dit was echter wel het geval met verdunde PEG. PP reperfundeerde de grote renale vaten, terwijl verdunde PEG zich diffuus verspreidde in de nier. We besloten dat PP eveneens in Thiels gebalsemde varkensnieren circuleert en dat nieren geschikt zijn voor dit onderzoek.

De volgende studie onderzocht drie aspecten **(hoofdstuk 9)**. Ten eerste wilden we ideale balsemcondities creëren en evalueren. Eén volledig varken en drie halve varkens werden meteen na euthanasie met een pomp onder gecontroleerde druk gebalsemd. Zo konden we een adequate en snelle weefselperfusie verzekeren. Inspectie van de verspreiding van de balsemvloeistof toonde dat eerst de arteriën werden gevuld, daarna de organen en uiteindelijk trad er veneuze drainage op. Herevaluatie na twee weken leerde dat de balsemvloeistof de weefsels verder had gekleurd. Dit betekent dat de verspreiding in de weefsels zich verderzette.

Nadien werd een langdurige reperfusie met PP van slagader tot ader aangelegd en werd de weerslag op de anatomie en de geschiktheid van de weefsels voor chirurgische training bestudeerd. In alle onderzochte modellen draineerde arterieel-geïnjecteerde PP via de veneuze canule. Ondanks reperfusie aan lage druk (30 mmHg) trad er lekkage op waardoor het debiet werd verlaagd eenmaal veneuze drainage optrad. De meeste organen vertoonden een adequate vulling van hun vaten. Vervolgens werd gedurende meerdere uren een reperfusie aangelegd die toeliet om chirurgische ingrepen te testen in realistische omstandigheden.

Ten laatste vergeleken we de microvasculaire reperfusie van PP in pompgebalsemde varkensdundarmen met menselijke dundarmen die met de zwaartekracht werden gebalsemd. PP stroomde in de haarvaten van pompgebalsemde varkensdarmen zonder uit de bloedvaten te treden en bereikte tenslotte de aders. PP werd echter geblokkeerd in zijtakken van kleinere slagaders van menselijke dundarmen. Vermoedelijk zijn bruine gefixeerde plekjes in de vaten van de mucosa hiervoor verantwoordelijk. De samenstelling van deze plekjes is ongekend. Uit dit onderzoek werd geconcludeerd dat drukgecontroleerde toediening van Thielse balsemvloeistof zo vlug als mogelijk na overlijden essentieel is om een goede balseming te hebben. PP lekt echter nog in variabele mate in verscheidene organen. Lange reperfusie is wel mogelijk aan lage debieten zonder dat dit een grote weerslag op de anatomie heeft zodat chirurgische ingrepen kunnen gebeuren.

Hoofdstuk 10 bevat voorlopige resultaten van experimenten op Thiels gebalsemd humaan weefsel. Hierbij werden voornamelijk nieren gereperfundeerd nadat deze verwijderd werden uit Thiels gebalsemde menselijke kadavers. We gebruikten dezelfde techniek zoals beschreven in hoofdstuk 8 maar de reperfusie met PP duurde 240 minuten. Gedurende de eerste 120 minuten werden statistisch significante gewichts- en volumetoenames gemeten. Nadien traden er geen statistisch significante veranderingen meer op. CT beelden toonden PP in de grotere niervaten tot en met vertakkingen aan de cortex. In de cortex waren echter hier en daar kleine ophopingen van contrast aanwezig die de interpretatie van de reperfusie daar bemoeilijkten. Vervolgens probeerden we de realiteit nog meer te benaderen door het debiet gedurende één

uur te verhogen naar 25 mL/min. Dit resulteerde in statistisch significante gewichts- en volumetoenames ondanks lage gemeten arteriële drukken.
Op een gelijkaardige manier werd één lever gereperfundeerd met een laag debiet en lage intravasculaire druk. Er werd een circulatie gecreëerd zonder opvallende weerslag op de anatomie van de lever. Meerdere kleine parenchymateuze contrastophopingen bemoeilijkten een duidelijke visualisatie van de vasculaire anatomie.
Op dezelfde wijze werd gedurende meerdere uren een circulatie aangelegd in één arm. Na 4u reperfusie vonden we in beperkte mate onderhuidse contrastophopingen. Op CT kon het verloop van de voornaamste arteriën en venen gevolgd worden tot in de hand. In de vingers konden we geen reperfusie aantonen. Vervolgens werden open vaatheelkundige technieken onder realistische omstandigheden getest.
Finaal probeerden we een langdurige reperfusie aan te leggen in een volledig Thiels gebalsemd kadaver. Hoewel we de vaten van organen en weefsels konden reperfunderen was er slechts lokaal veneuze drainage en geen uitstroom via de veneuze canule. Lokale variaties in arterioveneuze shunts en capillaire lekkage; microvasculaire obstructie; en klonters in de grote venen zijn potentiële oorzaken die een dynamische reperfusie in een volledig kadaver verhinderen.

Hoofdstuk 11 beschrijft in acht Thiels gebalsemde humane kadavers hoe via een transfemorale toegang een nieuwe aortaklep wordt geïmplanteerd ter hoogte van de natieve aortaklep. Eerst werd Paraffinum Liquidum (PL) retrograad in de arteria femoralis gepompt. Hierdoor werd de aorta gedilateerd waardoor via minimaal invasieve weg onder radioscopische controle de aortakleppen konden opgeschoven worden tot aan het hart. Radiologisch nazicht leerde dat vijf kleppen correct werden gepositioneerd. Dit gereperfundeerd model laat toe om endovasculaire transfemorale technieken te oefenen. Het beschreven model is wel eenvoudiger dan de hierboven besproken reperfusies omdat vulling van de venen niet beoogd werd.

Uit deze thesis kan besloten worden dat de betekenis van (niet)-gereperfundeerde humane kadavers als chirurgisch trainingsmodel nog onvoldoende gekend is. PP stroomt van slagader naar ader in Thielse varkens die met de pomp onder gecontroleerde druk werden gebalsemd. Deze langdurige reperfusie gebeurt aan lage drukken en gaat gepaard met lekkage die varieert van orgaan tot orgaan. Balsemen met de pomp is essentieel om latere reperfusie met PP te vergemakkelijken. Reperfusie leidt echter niet tot belangrijke anatomische vervorming zodat verscheidene chirurgische procedures kunnen gesimuleerd worden. In menselijke nieren, één lever en één arm die met de zwaartekracht werden gebalsemd stroomt PP ook onder toelaatbare intravasculaire omstandigheden en is het effect op de weefsels aanvaardbaar. Behalve in de dundarm werd het microvasculair verloop van PP in humaan Thiel weefsel nog niet onderzocht. De reperfusie van volledige Thiels gebalsemde menselijke kadavers staat nog

in zijn kinderschoenen en vereist dus nog onderzoek. Toekomstig onderzoek zal zich dus richten op pompgestuurde drukgecontroleerde verspreiding van Thielse balsemvloeistof in menselijke kadavers. Er zal ook onderzocht worden hoe de bloedvaten kunnen geledigd worden alvorens te balsemen. Bovendien moeten de reperfusie-eigenschappen van verscheidene perfusaten in Thiels gebalsemde lichaamsdelen vergeleken worden. Deze onderzoeken kunnen helpen om de reperfusie van slagader naar ader in volledige Thiels gebalsemde menselijke kadavers met een voorkeursperfusaat verder te ontwikkelen. Tenslotte kunnen we binnenkort chirurgische procedures op gereperfundeerde humane Thiel organen en extremiteiten aanbieden en evalueren.

Chapter 2

Introduction

General introduction

Until the 19th century, training surgeons - if any training existed at all - was through apprenticeships, meaning that students learned surgical practice through direct observation and by imitating the skilled teacher, both in the OR and in the clinical setting. Principles or guidelines for trainee selection, knowledge and practical skills learning and how to teach these, were lacking. Toward the end of the 19th century education became more structured by William S. Halsted who introduced a German-style residency surgical training system “see one, do one, teach one”, based primarily on graded responsibility.[3-5]

As stated above, this system remains the cornerstone of surgical training worldwide and has been successful in transferring knowledge and skills from one generation to the next. However, this model is no longer acceptable to either the surgical profession or to the well-informed public. Moreover, teaching and training opportunities have diminished by the introduction of the European Working Time Directive, increasing complexity of cases, new surgical technologies and therapies, growing importance of OR efficiency, intensifying demand for documentation and other “service-related duties”.[4] There is now more emphasis on enhancing the efficiency of the learning process. Furthermore, public demand for greater accountability and patient safety, have diminished opportunities for learning through work with patients.[6] As a result, interest in tools to teach surgical skills away from the patient in a structured fashion has increased dramatically. Surgical simulation shortens and flattens the learning curves of trainees resulting in error reduction and increased patient safety.[7-9] Simulation-based training is more and more integrated within training programs for clinicians at all stages and should serve as an adjunct to current surgical training practices.[10, 11] Residents would thus be trained in the laboratory until pre-set criteria have been met and only then would they be allowed to join procedures in the OR.[12] The scheduling practice also impacts on surgical skills acquisition. Residents retain and transfer skills better if taught in a distributed way instead of using short courses, which are assumed to be suboptimal.[13] Note that besides the type of training model, the curriculum in which it is implemented combined with formative feedback after the intervention are of significant importance to enhance skills acquisition.

This preface, firstly, describes the available tools to enhance surgical skills and emphasizes the lack of a prolonged ‘lifelike’ reperfusion from artery to vein in human bodies. Secondly, the development of Thiel embalmed human cadavers and use in various medical specialties is reported. Thirdly, a summary of pump-driven flow from artery to vein in fresh animal and human models is presented.

Surgical training models

Synthetic models

VT's are frequently used and have a basic design: a box with holes for trocars and for a camera or mirror displaying an image from a closed space, meant to simulate the insufflated peritoneal cavity.[14] Most manufactured VT's, irrespective of the technology used, have this basic design. Standard laparoscopic instruments can be employed to learn and practice basic surgical skills like individual maneuvers required during an operation (*e.g.* suturing, needle manipulation, tissue retraction, etc.), the sequence of tasks during a laparoscopic intervention or part of an operation (*e.g.* dissection of the gallbladder from the liver bed, dissection of the hilar structures of the gallbladder, etc.). Although VT's mostly utilize rubber or plastic parts to simulate tissues and anatomic relationships, fresh animal organs or organ groups may be used as well.

Synthetic endovascular training tools range from low-fidelity solid plastic models to high-fidelity devices with pulsatile flow and the addition of C-arm radiography.[15] These are relatively inexpensive, but they cannot fully simulate the dynamic flow of the human arterial circulation. Low-fidelity simulation is an effective method for training minimally invasive endovascular skills training and allows practicing essential early steps with force feedback. Deployment of single use devices is, however, wasteful and adds to the cost of training.

Thus, presently, a variety of synthetic simulators are available.[5] These models are safe, reproducible, portable, readily available and generally more cost-effective than animals or cadavers.

Virtual reality

VR technology has proven its potential for enhancing skills in laparoscopy, endoscopy and endovascular procedures.[5, 15] Most VR systems are commercially available and offer standardized tasks and multiple modules in various anatomical regions under realistic circumstances (*e.g.* laparoscopic cholecystectomy (CCE)).[16] In general, VR allows practicing more advanced surgical skills and entire procedures. Data can be captured and provide very detailed feedback of performance (*e.g.* precision, accuracy, error rates) using validated assessment metrics. Procedures or maneuvers can be repeatedly exercised until sufficient competence has been demonstrated using the real devices slightly modified over and over. Patient-specific simulations are also possible, which may allow repeated practice of a procedure before performing the real case. No ethical issues are related to the use of VR.

Limitations are significant cost, set-up, transport and maintenance cost. The devices are prone to technical failure and regular calibration is essential.

Animal models

Anesthetized animals, particularly the pig model, are commonly used in surgical training courses.[17] With respect to their anatomy and physiology, pigs constitute one of the best experimental animal models for human systems in surgical fields (*e.g.* urological, gastrointestinal, maxillofacial, plastic, transplantation surgery, etc.).[18-21] The dog is also one of the most used animals in experimental research and has a reasonable size to perform surgical procedures as laparoscopy, cardiac surgery, transplantation training and so forth and so on.[22-24] Furthermore, the surgical anatomy of sheep (*e.g.* liver, middle ear, gynecological structures, etc.) resembles that of humans, making them recommendable for training purposes.[25-27]

Live animal tissue offers excellent tissue manipulation and a high degree of realism for full procedure simulation in a wide range of surgical interventions.[5, 15] However, need for specialized staff to perform general anesthesia and purpose-built facilities for cleaning, storage and disposal are major disadvantages.[17] Moreover, the costs of running an animal laboratory are very high. In addition, practicing on animals is limited because of single-use, legal and ethical concerns (*e.g.* the Cruelty to Animals Act forbids the use of animals in the United Kingdom).[17] Also, although often limited, the anatomical and size differences compared to humans are significant limitations. For example, pig small intestines are used to demonstrate small bowel anastomosis, but the submucosal layer is difficult to identify when performing serosubmucosal anastomosis.[17]

Human cadaver models

The previously described models are all compromised by the lack or absence of normal human anatomical relationships and tissue handling.[7] Human cadavers are exceptional because complete operations can be performed under the most realistic conditions.[15] Others, however, criticize the high cost, availability limited to anatomical centers and for advanced procedural training, single-session use of endovascular tools, absence of validated assessment tools, as well as the often poor quality of cadaveric tissue.[5, 15] Currently, workshops are offered worldwide on fresh as well as embalmed human cadavers. Fresh cadavers can only be used for a short period, whereas soft embalmed bodies (*e.g.*, Thiel cadavers) are valuable alternatives due to their prolonged and realistic preservation. In 2011, Gilbody et al. systematically reviewed the use of human cadaver workshops for training postgraduate surgical trainees in basic or advanced surgical skills.[7] Included studies needed to have a clearly defined outcome measure to evaluate the efficacy of training. Eight studies were identified. Two studies attempted to assess transfer of skills acquired during cadaveric training to the clinical setting. None showed

any evidence that skills learnt by training on cadavers improved performance in the OR. The absence of a control group, low number of participants and mainly inexperienced residents involved in both studies may explain this finding. Three studies used subjective questionnaires and revealed that trainees valued cadaveric training. Together, both trainees and assessors believe that cadaver workshops are useful adjuncts when teaching surgical skills, but there is currently limited evidence for the effectiveness of cadaver workshops in surgical training.

Likewise, vascular reperfusion of human cadaver models has been poorly studied in terms of acquired skills and impact on skills transfer to real life.[28-30] Moreover, a more advanced type of human cadaveric circulation, which is the prolonged dynamic lifelike reperfusion from artery to vein has not yet been described in literature. Some of the advantages and disadvantages of various models are summarized in table 1.

Table 1. *Types of simulations available. Adapted from Reznick et al.*[5]

Simulation	Advantages	Disadvantage	Best use
Bench models	Cheap, portable, reusable, minimal risks	Acceptance by trainees, low fidelity, basic tasks, not operations	Basic skills for novice learners, discrete skills
Live animals	High fidelity, availability, practice hemostasis and entire operations	Cost, special facilities and personnel required, ethical concerns, single use, anatomical differences	Advanced procedural knowledge, procedures in which blood flow is important, dissection skills
Cadavers	High fidelity, only “true” anatomy simulator, practice entire operations	Cost, availability, single use, compliance of tissue, infection risk	Advanced procedural knowledge, dissection, continuing medical education
VR simulators	Reusable, data capture, minimal setup time	Cost, maintenance, and downtime, acceptance by trainees, three dimenstions not well simulated	Basic laparoscopic skills, endoscopic and transcutaneous procedural skills

Thiel embalmed cadavers

Embalming fluids

Until now, formaldehyde-based embalming has been the most widely used preservation method worldwide (*i.e.* 87 % of centres).[31] Formaldehyde fixes the tissues and quickly stops all decomposition processes of the corpse.[32] Moreover, it destroys pathogens in the body through disinfection and sufficient concentrations prevent microbiological proliferation on preserved cadavers. Despite these excellent properties, high concentrations of formaldehyde or prolonged usage are associated with important hardening and tissue discoloration. Also, this product has a pungent, irritating odour, making it unpleasant to work with. Moreover, formaldehyde is highly toxic to all animals, regardless of method of intake.[33] The International Agency for Research on Cancer reclassified formaldehyde as a known human carcinogen.[34] A saturated water solution of 37 % formaldehyde by mass is called 100 % formalin.

Development of the original Thiel embalming

In 1992, Professor Walter Thiel from Graz, Austria, reported an entirely new soft-fix method with a minimal concentration of formalin.[32] This method was developed over a period of 30 years (1960 - 1990). Initially, numerous combinations of solutions with different concentrations of substances were tested using *in vitro* series of fresh beef. Preservation of both natural tissue color and softness were of particular importance. The *in vitro* experience was gradually implemented to develop the final Thiel embalming solutions. Lowering the concentration of formalin was essential during this process, but tissue preservation and tissue hardening effect of formalin needed to be balanced.

In total, 977 complete human bodies and many cadavers after autopsy were tested. Thiel observed that concentrations of 3 % formalin still caused tissue hardening and discoloration, while concentrations down to 2 % were not always associated with optimal cadaver preservation. Hence, from 1965, boric acid for disinfection (one of the final components) was added, among others (that were later omitted). In 1972, a stem solution of ammonium nitrate-chlorcresol mixture with or without potassium nitrate was successfully implemented. Later, sodium sulphite and morpholine became final components, due to their favorable effect on color preservation. In 1986, ethyl alcohol was omitted from the embalming solution because of its flammability and replaced by mono-ethylene glycol for preservation of tissue plasticity. Eventually, formalin, ammonium nitrate, boric acid, chlorcresol, sodium sulphite and morpholine became the main components of the embalming solutions.

Administration of this mix of products results in an embalming that meets high standards of preservation. The color and consistency of the tissues are very close to those of living individuals and produces flexible cadavers that are preserved for a long period. The embalming solutions are largely odorless and cause no irritation of skin and mucosa. Moreover, the fluids remain stable during prolonged storage and effectively disinfect the cadavers without mold formation. Concentrations of formaldehyde in room air remain under the limit of detection. The excellent quality of preservation is visualized in a photographic atlas of practical anatomy published by Thiel in 1997.[35]

Thiel embalming is still relatively unknown.[31] The main obstacle to its wider use is probably the language barrier, as publications describing the technique are in German. Negative aspects are the long preparation time and the high cost of the procedure (material, chemical products). At Ghent University, Thiel embalming costs approximately € 760, while the price for a classic formalin-based technique is only € 30. If the infrastructure (*e.g.* embalming bath, vacuum sealing to preserve the bodies, etc.) is also included, prices go up to $ 1,200.[36]

Composition of the embalming fluids

The original embalming procedure is quite complicated and, as described above, consists of several solutions. Table 2 shows the formulae of the embalming solutions as described by Thiel in 1992.

Table 2. *Composition of the original Thiel embalming fluids. Adapted from Thiel.*[32]

Stem solution 1989	**Amount**
Hot tap water	63.30 %
Boric acid	1.90 %
Mono-ethylene glycol	19.00 %
Ammonium nitrate	12.6 %
Potassium nitrate	3.2 %
Stem solution 86/3 (chlorcresol)	
Mono-ethylene glycol	90.90 %
4-chloro-3-methylphenol	9.10 %
Perfusion solution 1989	
Stem solution 1989	14.30 L
Stem solution 86/3 (chlorcresol)	0.50 L
Sodium sulphite	0.70 kg
Formalin	0.30 L
Tank solution 1986	
Hot tap water	71.90 %
Boric acid	2.16 %
Mono-ethylene glycol	7.19 %
Ammonium nitrate	7.19 %
Potassium nitrate	3.60 %
Formalin	1.44 %
Sodium sulphite	5.00 %
Stem solution 86/3 (chlorcresol)	1.44 %
Visceral solution 1989	
Stem solution 1989	10.00 L
Stem solution 86/3 (chlorcresol)	0.50 L
Sodium sulphite	0.50 kg
Morpholine	0.30 L
Formalin	0.85 L
Isopropyl alcohol	3.00 L
Brain-spinal cord solution 1990	
Tap water	40.0 %
Mono-ethylene glycol	10.0 %
Isopropyl alcohol	40.0 %
Formalin	10.0 %

Administration of the embalming solutions

The embalming procedure starts shortly after arrival of the body. Originally, Thiel reported a complex embalming method that consists of stepwise perfusion of the cadaver followed by immersion in a bath.

Perfusion solutions

These are administered over three days (table 3). Primarily, the superior sagittal sinus is cannulated after making a small hole drilled through the skull. This is followed by tracheal intubation. Two cannulas are then inserted in the external iliac artery in antegrade and retrograde direction. Next, the colon is embalmed transrectally with visceral solution and the stomach is filled with the same fluid using a gastric tube. Afterwards, visceral solution is administered in the superior sagittal sinus at 150 mmHg. Subsequently, the lungs are filled with the same fluid through the tracheal tube. Then, the perfusion solution is injected at 150 mmHg through both cannulas placed in the external iliac artery. Lastly, the brain-spinal cord solution is administered through two lumbar puncture needles, which are punctured transnasally through the lamina cribrosa. After perfusion, the cadaver typically has a bloated appearance.

Table 3. *Administration of the individual embalming fluids.*

Cannulation	Type of embalming solution	Amount	Remarks
Superior sagittal sinus	visceral solution 1989	9.0-10.0 L	administered in 2 steps
Trachea	visceral solution 1989	2.5 L	cannula clamped until complete brain embalming
Stomach	visceral solution 1989	1.0 L	
Rectum	visceral solution 1989	4.0 L	
External iliac artery	perfusion solution 1989	11.0-15.0 L	volume dependent of body size
Lamina cribrosa	brain-spinal cord solution 1990	not reported	procedure often repeated

Immersion solution

Afterwards, the cadaver is immersed in a hypertonic tank solution for at least 6 months. In the first days after perfusion, some bluish and reddish discoloration of the skin often occurs. This slowly disappears and the skin increasingly pales.[37] Authors assume this is displaced blood that was not drained during perfusion. Due to osmosis, the cadaver gradually loses fluid and regains its initial appearance.[32] The color of the fluid in the bath turns increasingly brown over time, suggesting that blood cells are broken down and dissolve into the bath fluid. However, these advantageous effects (*i.e.* water loss and passage of blood toward the tank) can be associated with mummification of the hands and feet. This phenomenon occurs only if the embalming solution did not reach these parts of the cadaver and can be prevented by local intravascular injection of perfusion solution before immersion in the bath. In addition, the epidermal layer detaches during immersion in the tank. It is still unknown whether the whole epidermis or only one or a few epidermal sublayers get lost. Although not tested by Thiel, the author stated that this can be prevented by short immersion of the cadaver in high concentrated aqueous formaldehyde solution.[32]

Care of the bodies during the embalming process

Dehydration of the cadavers during preparation can be halted through wrapping them in moistened wipes and occasional wetting of exposed regions with an appropriate solution (table 4).

Table 4. *Composition of wetting solution T86. Adapted from Thiel.*[32]

Components	Amount
Hot water	87.72 %
Boric acid	2.63 %
Mono-ethylene glycol	4.39 %
Sodium sulphite	4.39 %
Stem solution B	0.87 %

This solution provides an additional protection against mold formation and does not interfere with the tissue colour. Moreover, this technique can be successfully applied if the embalmed cadaver suffered significantly due to intensive use and in case of no further preservation in the tank.

Preservation of the bodies

Cadavers can be sealed in plastic bags or remain in submersion. In both cases gradual fluid loss occurs.

Improved variant of the original embalming solutions

In 2002, Thiel published an improved variant of his original embalming procedure.[38] Although several adjustments were implemented, this variant mainly focused on preservation of the brain and spinal cord (table 5 and 6).

Table 5. *Adjustments observed in the improved embalming technique.*

Added	Solution
Mono-propylene glycol	stem solution I and II tank
Ethyl alcohol	brain-spinal cord solution intestinal solution perfusion solution tank
Morpholine	perfusion solution
Increased concentration	
Formalin	brain-spinal cord solution intestinal solution perfusion solution
Omitted	
Mono-ethylene glycol Isopropyl alcohol	

Table 6. *Composition of the improved variant of the original Thiel embalming solution. Adapted from Thiel.*[38]

Stem solution I 1998	**Amount**
Hot tap water	63.30 %
Boric acid	1.90 %
Mono-propylene glycol	19.00 %
Ammonimum nitrate	12.60 %
Potassium nitrate	3.20 %
Stem solution II 1998 (chlorkresol)	
Mono-propylene glycol	90.90 %
4-chloro-3-methylphenol	9.10 %
Perfusion solution for one human cadaver 2001	
Stem solution I 1998	12.00 L
Stem solution II 1998 (chlorkresol)	0.50 L
Sodium sulphite	0.60 kg
Morpholine	0.450 L
Formalin	0.50 L
Ethyl alcohol	2.00 L
Tank solution 1998	
Hot tap water	65.47 %
Boric acid	2.16 %
Mono-propylene glycol	7.19 %
Ammonimum nitrate	7.19 %
Potassium nitrate	3.60 %
Ethyl alcohol	6.47 %
Formalin	1.44 %
Sodium sulphite	5.00 %
Stem solution II 1998 (chlorkresol)	1.44 %
Visceral solution 2001	
Stem solution I 1998	12.00 L
Stem solution II 1998 (chlorkresol)	0.50 L
Sodium sulphite	0.60 kg
Morpholine	0.450 L
Formalin	1.00 L
Ethyl alcohol	2.00 L
Brain-spinal cord solution 1998	
Tap water	40.00 %
Ethyl alcohol	45.00 %
Formalin	15.00 %

Firstly, in the original embalming method, Thiel observed that despite the presence of 10 % formalin in the brain-spinal cord solution, the central nervous system turned in an almost pulpy condition. Removing mono-ethylene glycol from this solution had only a slight effect on the organs' constitution. The small cerebrospinal fluid volume (5-6 %) in relation to the brain size and the difficulty to replace this fluid by embalming solution can explain this observation. To

improve the preservation of brain and spinal cord, these structures were embalmed with a peristaltic pump for 24 hours. Therefore, two lumbar puncture needles were passed through the lamina cribrosa into the anterior horn of the lateral ventricles and a third lumbar needle was inserted between L5 and S1 into the subarachnoid space, enabling a continuous circulation from head to spinal cord at 67.2 mL/min.

Secondly, adding ethyl alcohol to all embalming solutions (except stem solutions I and II) or its use instead of isopropyl alcohol improved tissue solidity without loss of full natural elasticity. This improvement was particularly observed in the brain and in the fat tissue.

Thirdly, in 1998, the slightly toxic and corrosive mono-ethylene glycol was replaced by the unaggressive and non-toxic mono-propylene glycol. The latter product impacted on tissue color preservation, which was balanced by adding 3 % morpholine.

Fourthly, the concentration of formalin in the perfusion solution was increased to enlarge the strength of the tissues, but also influenced muscle color. Adding morpholine largely compensated this phenomenon.

Simplified embalming procedure

Currently, centres (*e.g.* Dundee, Ghent, Graz and Zurich) adopted a more simplified approach that consists of vascular embalming and subsequent immersion in the tank because additional infusions seem superfluous for bodies used for surgical training.[37, 39-41]

Vascular access

The initial step remains vascular embalming, but the access among centres varies. Although Thiel employed the external iliac artery, other vessels can be cannulated more easily. Presently, at the institute of anatomy in Ghent, Belgium, the femoral artery is the only and preferred access.[39] The femoral vein is not cannulated for embalming or venous drainage of blood. If the femoral artery is unsuitable, the carotid artery or great saphenous vein is being used. Similarly, Groscurth et al. from the institute of anatomy in Zurich, Switzerland, prefer one arterial access.[40] At the Centre for Anatomy and Human Identification in Dundee, United Kingdom, infusion is done via the femoral artery and via the superior sagittal sinus.[37] This double approach is currently the same as in Graz, Austria.[37]

Embalming techniques

A few centres still use the original perfusion solution 1989 and tank solution 1986 and administer 15.8 L perfusion solution for an average size cadaver.[39, 40] However, presently, as practice has gradually evolved over time, formulae and perfusion volume used in Graz and Dundee are slightly different than the most recent version published by Thiel in 2002.[37] This means that the perfusion and tank solutions still have the same components (except for ethyl alcohol that is not used in the tank solution anymore), but in a slightly different concentration.

Moreover, as stated above, these centres use a double vascular access and administer 13.0 L and 5.5 L in the arteries and veins, respectively. Furthermore, storage time in the tank is now considerably shorter compared to the original publication by Thiel (table 7). In the literature, however, the impact of these measurements on the embalming quality has not been described in detail. Afterwards, the bodies are stored in sealed plastic bags until use. Cadavers that are in active use are stored in the tank between sessions. Table 8 shows the composition of Thiel embalming fluids as used in Dundee and Graz. Note that no toxicity data concerning these mixtures are presently available.

Table 7. *The simplified embalming procedures applied in several centres.A: artery; V: vein; * embalming formulae and double vascular access are currently the same as in Graz.*[37]

Author	Centre	Perfusion solution	Volume	Vascular access	Immersion solution	Duration in tank	Storage
Groscurth *et al.*[40]	Zurich, Switzerland	perfusion solution 1989	15.8 L	femoral or carotid artery	tank solution 1986	4 weeks	plastic bags
Kerckaert *et al.*[39]	Ghent, Belgium	perfusion solution 1989	15.8 L	femoral artery	tank solution 1986	4-6 weeks	plastic bags
Eisma *et al.*[37] *	Dundee, UK	adjusted perfusion solution 2001	A: 13 L V: 5.5 L	femoral artery and superior sagittal sinus	tank solution 1998	2 months	plastic bags

Table 8. *Stem solution II contains 9.10 % 4-chloro-3-methylphenol and 90.90 % mono-propylene glycol. Total volumes are approximate and based on an average size cadaver and the size of the tank used in Dundee. Adapted from Eisma et al.*[37]

Components	Arterial infusion	Venous infusion	Tank solution
Hot tap water	6.8 L	1.45 L	1250 L
Boric acid	250 g	80 g	45 kg
Mono-propylene glycol	2.5 L	780 mL	150 L
Ammonium nitrate	1,680 g	520 g	150 kg
Potassium nitrate	420 g	130 g	75 kg
Stem solution II 1998	500 mL	190 mL	30 L
Sodium sulphite	700 g	190 g	105 kg
Morpholine	150 mL	110 mL	-
Formaldehyde (8,9 %)	2.1 L	1.5 L	125 L
Ethyl alcohol	1 L	1.1 L	-
Total volume	13 L	5.5 L	1,720 L

Flexibility of Thiel cadavers

The notable flexibility of Thiel cadavers creates many new applications, for example, surgical procedural training is generally favoured for its realism.[39, 42-48] However, as discussed below, the tissue integrity of fresh cadavers more closely approaches the living and thus remains the golden standard for biomechanical research. Benkhadra et al. investigated the pronounced suppleness of Thiel cadavers.[49] Light microscopic analysis of the biceps brachii muscle and brachioradialis tendon fibres of Thiel cadavers was compared with that of fresh cadavers and formalin-preserved cadavers. The muscle fibres of Thiel cadavers had a cut-up 'minced' appearance, but were contained in an intact collagen sheath. These changes were not found in the other cadavers. Probably, corrosive effects of boric acid cause fragmentation of muscles proteins, which could explain the suppleness of Thiel cadavers. This study did not evaluate alternative explanations for the observed flexibility such as electro-microscopic assessment of possible alterations in collagen ultrastructure.

Applications for Thiel cadavers

As stated earlier, in contrast with Thiel cadavers, conventional embalming procedures using mainly formalin have far less applications due to profound changes of color; altered biomechanical tissue properties by extensive protein cross-linking; fragility of organs and tissues; and formaldehyde's known toxicity and annoying odour.[37, 40, 50] Table 9 compares formalin-based embalming with Thiel embalming.

Table 9. *Formalin-based embalming versus Thiel embalming.*[31, 32, 36, 37, 51]

Formalin-based embalming	Thiel embalming
tissue rigidity	soft tissues
tissue discoloration	natural tissue colors
tissue dehydration	bloated appearance followed by gradual dehydration
tans tissues	preserved tissue integrity
rapidly coagulates blood	no data available
constricts capillaries	no data available
unpleasant odor	largely odorless
deteriorates with age	stable solutions during long storage
most widely used	relatively unknown
long preservation	long preservation (± 1 year)
preservation in open-air	preservation in sealed bags or embalming bath
bactericidal, fungicidal and insecticidal	effective cadaver preservation
relatively limited applications	many applications
suitable for dissection	more difficult dissection
cheap	expensive and complex

Anatomical training model

Because of the prolonged preservation, Thiel cadavers can be employed to teach anatomy during dissection courses.[52] Others, have suggested that Thiel cadavers are less suitable for dissection and only use them for surgical training because tissue dissection is more difficult compared to formalin-fixed bodies.[31, 37, 39] For example, during dissection, yellow oily liquid collects in the tissue planes. This is thought to be the lipid from disrupted fat cells.

Proponents of anatomical training on this model argue that the good preservation of tissue planes, the ease of skin removal and superficial fascia dissection lead to easier and faster separation of superficial structures. Moreover, for teaching the musculoskeletal system, Thiel cadavers are superior. Tissue flexibility allows a lifelike demonstration of joint mobility and movements of the forearm aid in the identification of muscles. The abdominal and thoracic organs are often very realistic. Due to their pliant structure, organs can be more easily displaced, which facilitates overall access and visualization of body structures. Features seen in removed Thiel embalmed-organs are very similar to the *in vivo* condition. Also, vessels are soft and flexible and can be subjected to latex injection.[53] The relatively poor preservation of the brain and bone marrow; soft texture of the uterus; and detachment of the epidermal layer of the skin are drawbacks.[31, 52]

Surgical training model

Thiel cadavers afford working conditions almost as realistic as in the living and have been widely appraised for hands-on workshops for several surgical disciplines.[39, 42-48] Thiel embalmed tissues remain in good condition, in spite of repeated use during surgical procedures. Workshops can be offered on the same cadaver for one year.

Laparoscopy

In 1996, Ablaßmaier et al. reported the first laparoscopic surgical procedure (*i.e.* Billroth II gastrectomy) performed on seven Thiel cadavers and praised the good training possibilities to simulate minimally invasive surgery.[54] Giger et al. published a qualitative assessment of laparoscopic procedures (*i.e.* colon, hernia, vascular and bariatric surgery) on Thiel cadavers using a five-point Likert scale and underlined that authenticity of tissue color, tissue consistency and operative tactility were scored highly (mean ≥ 4).[42] The lowest rates were recorded during the vascular course (*i.e.* laparoscopic infrarenal aorto-bifemoral bypass) because training occurred on non-perfused blood vessels. Rai et al. evaluated laparoscopic nephrectomy on Thiel cadavers and all steps of this procedure were rated 4.0 plus by trainees and faculty members.[55] Research evaluating transfer of laparoscopic skills from the Thiel cadaver to real life is still lacking.[42]

Flap raising and microvascular surgery

Wolff et al. established training courses on Thiel cadavers and confirmed their suitability to learn flap raising.[43] Pedicles at free flap donor sites are perfectly preserved and even perforators can be dissected and exposed. Vessel lumina can be rinsed. Nerves are slightly weaker than in fresh cadavers. This is merely an observation and is not further investigated in this study. Typical characteristics of arteries and veins can be distinguished microscopically, allowing realistic handling.

Endoscopy

The usage of Thiel cadavers for cystoscopy and transurethral resection of the prostate has been explored with promising outcomes.[36] These preliminary findings need, however, further validation.

Cardiac surgery

This will be discussed in **chapter 11** of this thesis.

Transplant surgery

Cabello et al. published their initial experience in organizing workshops in kidney prelevation and transplantation and stressed that this procedure can be performed as in real life.[56]

Other surgeries

Thiel cadavers are preferable to practice thyroid surgery and to learn ultrasound-guided percutaneous tracheal puncture.[44, 45] Despite a success rate of 90 % for tracheal puncture, damage to the thyroid was present in eight out of nine successful punctures, underscoring the relevance of training. Thiel cadavers provide lifelike circumstances to perform various surgical techniques on external and middle ears, but also to practice oral surgery and implantology.[46, 47]

Other applications

Development and refinement of techniques

Because length and diameter of vessels, nerves and muscles are similar as *in vivo*, Thiel cadavers are feasible to study the anatomy of dissected flaps (*e.g.* the free vascularized sural nerve graft combined with a fasciocutaneous posterior calf flap pedicled on the superficial sural artery).[57] The anatomical findings are useful to assess the suitability of these flaps for nerve reconstruction and soft tissue coverage in case of extended nerve and soft tissue defects of the extremity. Thiel embalmed arteries are particularly suitable to investigate microvascular anastomoses in terms of vessel patency, leakage and stricture using angiography; and vessel wall structure using light microscopy and scanning electron microscopy.[58] In 2014, Wolff et al. described reperfusion of Thiel embalmed flaps from artery to vein to practice flap raising procedures.[59] Reperfusion was established with a thin aqueous solution, which easily extravasates. As a consequence, venous return was only observed in a small number of flaps or even missing completely. Sufficient venous return in a flap is essential to assess the quality of flap raising and subsequent functioning. This is impossible in this model, limiting its reliability and underlines the relevance of using a better perfusate.

Technical feasibility of challenging surgery has been tested on Thiel cadavers. For example, hybrid natural orifice transluminal endoscopic approach for Roux−en−Y gastric bypass[60], transanal ileoproctostomy[61] and anorectal transplantation[62]. Moreover, new techniques in orthopedic and trauma surgery can be tested.[63-65] The almost natural joint mobilization allows

undertaking morphometric studies and may improve technically challenging surgery (*e.g.* latissimus dorsi tendon excursion).[66] Furthermore, Thiel cadavers have been employed to compare emergency cricothyroidotomy kits.[67]

Thiel cadavers have proved advantageous in the development and modification of vertical obturator nerve block, selective peripheral and pectoral nerve block and axillary brachial plexus block.[68-72]

Images of inflated Thiel embalmed lungs are of high quality, simulating chest radiographs of patients and were used for the analysis of the image quality performance of digital radiography systems.[73] Thiel cadavers serve as a clinically relevant phantom for cone-beam computerized tomography acquisition used in daily practice for setup correction in multiple radiotherapy domains.[74] Moreover, based on anatomically validated Thiel cadaver datasets, new guidelines for brachial plexus delineation have been developed, which may lead to diminished upper extremity symptoms after radiation therapy.[75, 76] In addition, there is growing interest in Thiel cadavers as a model for MR-guided interventional procedures.[77]

Biomechanical research

Freezing is currently the standard postmortem storage method for the study of the mechanics of biological systems. Fessel et al. evaluated the biomechanical characteristics of fresh-frozen and Thiel embalmed human digitorum profundus tendons.[50] Thiel embalmed tendons had statistically significant (P = 0.048) lower failure stress (probably due to collagen denaturing) and did not faithfully reflect biomechanical properties of fresh frozen tendons. The authors recommended against the use of Thiel embalmed tendons for biomechanical research. Unger et al. investigated the effects of Thiel embalming on cortical bone mechanics and observed after six months of Thiel storage a reduced elastic modulus.[78] Likewise, in biomechanical studies investigating failure loads of orthopedic implants, fresh-frozen specimens are preferable. These findings contrast with several reports, underscoring preserved native tissue properties of Thiel cadavers.[43, 46, 47, 71, 79] Thiel embalming diminishes tendon and bone mechanical properties, but macroscopic tissue integrity remains sufficient to facilitate handling and anatomical research.[50] Thus, the mechanical effects of Thiel embalming on connective tissues are complex because of softening effects of boric acid and the cross-linking effect of formalin. Perhaps, the former predominates.

Besides, Thiel cadavers enable the study of human middle ears mechanics in response to bone conduction stimulation.[80]

Varia

Eisma et al. investigated if respiration-related movement of the liver during ventilation in Thiel cadavers has potential for research and training in minimally invasive procedures.[77] The applied ventilation is comparable to tidal volumes at rest and the liver displacement is similar to

literature. Thiel-embalmed tissue is not suitable for radiofrequency ablation studies.[81] The electrical conductivity of Thiel embalmed liver exhibits a frequency response similar to radiofrequency tumor ablation, but this is much higher than obtained from *ex vivo* fresh porcine liver. As a result, the radiofrequency ablation zone of a Thiel-embalmed liver sample is extremely small due to its much higher conductivity.

Advantages and disadvantages of Thiel embalming

Table 10. *Advantages and disadvantages of Thiel embalming.*

Advantages	Disadvantages
soft-tissue preservation	pulpy brains and spinal cord
lifelike preservation of tissue colour and consistency	often inexplicable tissue quality variation within and between bodies
low formaldehyde concentration	epidermal layer detachment
effective disinfection	dehydration during storage in open air
largely odourless	storage in sealed bags or in tank
causes no skin or mucosa irritation	long and complex preparation
stable embalming solutions during long storage	embalming technique varies between centres
teaching musculoskeletal system	high procedural and infrastructural costs
surgical training model	less suitable for anatomical dissections and biomechanical research
multiple applications	relatively unknown

Dynamic postmortem reperfusion of animal and human models

Since the beginning of the 16th century, researchers investigated the human vascular anatomy and tried to visualize the vascular system postmortem.[82, 83] According to injection materials, the techniques can be classified into six groups: (I) corpuscular preparations in gelatin or agar, (II) corpuscular preparations in watery solution, (III) oily liquids, (IV) hydrosoluble preparations, (V) casts and (VI) miscellaneous.[83] Most classic methods only perfused single organs. Few studies described angiographies of complete human corpses and were, generally, applied on human embryos and fetuses or newborns directly after death. Mostly, contrast agents were injected with a syringe.[84, 85]

Below, a summary of dynamically reperfused animal and human models with the aim of surgical training (fresh pig organs) and postmortem diagnostics (forensic medicine) is presented. Dynamic reperfusion is defined as 'lifelike' ongoing pump-driven circulation of a perfusate from artery to vein. Note that this definition focuses on the proper course of the perfusate. Arterial pulsations are not mandatory.

Animal models

Fresh

In 1993, Szinicz et al. described pulsatile perfusion of fresh pig organs and organ groups (*e.g.* liver and gallbladder; heart and lungs; stomach and intestines) for laparoscopic surgery simulation.[86] This reperfused model is currently widely used in laparoscopic VT courses. The organs are placed in a commercially available box, containing two tubes inserted through the sidewalls. The arterial tube serves as inflow of the specimen. Another tube is placed at the lowest point of the box as the outflow from the venous system. A specially adapted pulsating pump delivers a mixture of tap water and red dye into the arterial tube for several hours. Multiple surgical procedures can be simulated on this model (*e.g.* CCE, hepatic resection, intestinal resection and anastomosis, controlling bleedings, etc.). The presence of pulsations is a major advantage, but the low viscosity of tap water (*i.e.* 1.0 Millipascal.second (mPa.s) at 20°C) and the reperfusion at pressures ranging between 150 and 180 mmHg will rapidly lead to extravasation of fluid. Keep in mind that the mean arterial blood pressure in adult pigs is 90 mmHg (unpublished data). Subsequently, deformation will be significant and venous reperfusion is limited. Last but not least, postmortem reperfusion is only possible until ten hours after death and storage time of fresh tissues is short.

In 2006, Grabherr et al. conducted the first truly dynamic angiography or the so-called 'postmortem circulation' from artery to vein, in whole and hemibodies of a cat and dogs.[87] In a feasibility study, diesel oil was injected with a roller pump, installing a dynamic reperfusion for approximately one hour. A fat-soluble contrast agent Lipiodol (*i.e.* Lipiodol Ultra Fluide®) was injected as a bolus during ongoing reperfusion and angiography was performed. The vascular system was visualized up to the level of smaller supplying and draining vessels and was comparable to clinical angiographies, but perfusion parameters such as flow rate and vascular pressure were not described. Additionally, microvascular flow of diesel oil was evaluated in the chicken chorioallantoic membrane with fluorescein isothiocyanate dextrane and revealed that capillaries were not entered, suggesting flow across arteriovenous shunts. The aim of this research was to develop a minimally invasive autopsy method, by injecting contrast-enhanced perfusate in the vascular system and performing a CT. As a result, postmortem diagnoses can be made before a classical autopsy procedure is performed. Leakage into the gastrointestinal lumen does occur with this new technique. Note that the viscosity of the perfusate is a determinant of the level of microvascular reperfusion and thus indirectly leakage.

Human models

Forensic medicine

In postmortem investigations, minimally invasive angiography permits the detection of vessel abnormalities and bleedings and may be an adjunct or a replacement of the traditional autopsy.[87] The perfusates used to reperfuse a body can be broadly divided into oily and aqueous solutions.[82, 87] The former are more confined to the vessel lumen, while the latter quickly extravasate and accumulate in the interstitial space. Note that increased capillary wall permeability in the early postmortem phase facilitates leakage. Therefore, fat-soluble perfusates and high-molecular weight water-soluble molecules are preferable. Moreover, only oily perfusates will rinse postmortem clots. At present, dynamic reperfusion has only been established with oily perfusates.

In 2008, Grabherr et al. first visualized the three phases of the vascular system (*i.e.* arterial, parenchymatous and venous).[88] They elaborated the two-step postmortem angiographic technique and reperfused the upper (n = 2) and lower (n = 2) extremities of fresh human cadavers with a modified heart-lung machine that contained a pressure-controlled and volume-controlled perfusion mode. The pump circulated PP diluted with 20 % decane, which was rendered visible by addition of Sudan red III. The final viscosity of diluted PP was 24.4 mPa.s at 20°C, which is substantially higher than the intrinsic viscosity of vital blood (4-5 mPa.s).[89]

Firstly, antegrade arterial reperfusion of the lower extremities was initiated for three minutes through a cannula introduced into the femoral artery. Reperfusion for five minutes was

established with the following parameters: inlet pressure approximately 50 mmHg and flow rate approximately 200 mL/min. After three minutes of circulation, a bolus of 40 mL Lipiodol was injected into the arterial system and reperfusion was visualized with multi-detector CT angiography. Vascular reperfusion was established without edema or absorption of PP and enabled drainage of remaining blood through the tube in the femoral vein. Both arteries and veins were visible and allowed them to make vascular diagnoses without autopsy.

Secondly, sole retrograde venous reperfusion of the upper extremities was installed because venous lesions were suspected. Therefore, the brachial vein was cannulated and reperfusion was installed for five minutes at a flow rate of 200 mL/min and inlet pressure of 30 mmHg. In the first case, a bolus of 40 mL Lipiodol was injected after one minute of reperfusion and multi-detector CT angiography was performed. In the other case, 20 mL of a 1:4 mixture of Lipiodol and PP was injected as a bolus during ongoing circulation. In both cases, sole venous reperfusion enabled to detect venous pathology such as local pinpricks and periodic narrowing of the lumen in the context of drug abuse.

Later, the same author further improved this model, resulting in a standardized protocol for high-quality postmortem angiography in human cadavers.[90] This protocol facilitates radiologic interpretations by decreasing artifacts during reperfusion and by using high perfusion volumes that completely filled the vessels.

Another landmark was the introduction of a new oily contrast agent Angiofil® (Fumedica AG, Muri, Switzerland), mixed with PL. Reperfusion with 6 % Angiofil® mixture yielded the best radiological opacity of the entire vascular tree. In order to obtain these images, the femoral vessels were cannulated in a caudal-to-cranial direction on one side. Then, the standardized protocol was applied, which includes an initial filling of the arterial system, followed by venous reperfusion and a short lifelike circulating phase (table 11). While developing this model, the authors observed that by sole arterial injection veins were also visualized but separate filling of arteries and veins resulted in a better filling of the vascular system. No accumulation of PL was observed in the body cavities during autopsy, which can be explained by the higher viscosity of PL compared to PP. Hence, PL is recommended during whole-body reperfusion as extravasation in the gastrointestinal tract hardly was noted.

Table 11. *Resulting protocol. Adapted from Grabherr et al.*[90]

Application	Flow rate (mL/min)	Duration (min/s)	Total volume (mL)
Arterial phase	800	1'30"	1,200
Venous phase	800	2'00"	1,600
Dynamic (circulating) phase	200	2'30"	500
Set filling (3/8"-tube)			400
Total consumption			3,700
Preparation		**Volume (mL)**	
Paraffin oil		3,500	
Angiofil (6%)		210	
Total mixture		3,710	

Surgical research

Multiple studies have tested surgical techniques in human cadaver tissues, using various reperfusion methods, but long dynamic reperfusion in Thiel cadavers has not been reported, underscoring the importance of the objectives of this thesis.[91-97] Installing a long dynamic reperfusion in a whole body is unique because various types of advanced surgical procedures can be performed on reperfused arteries and veins. Moreover, the vessels remain filled and bleedings persist - if not anticipated - during surgical procedures, lasting for several hours.

Chapter 3

Rationale and objectives

In 2005, a delegation of the departments of anatomy and surgery from Ghent University hospital visited the University of Fribourg, Switzerland, to learn the Thiel embalming technique.[39] In the summer of 2006, a surgical training center with Thiel cadavers was officially opened in Ghent as the second in Europe. Both clinicians and anatomists work together and offer numerous workshops nationally and internationally to practice advanced surgical procedures such as laparoscopic liver resections, bariatric surgery, colorectal procedures, minimally invasive thoracic surgery, orthopedic surgery and so forth.

Although performing surgery on Thiel embalmed human tissues is certainly highly realistic, it remains a great challenge to further elaborate this embalmed model by the reperfusion from arteries to veins. Establishing vessel circulation may cause surgical bleedings during surgery, forcing surgeons to dissect carefully as during live surgery. Secondly, the embalming technique and the spread of the embalming mixture will be evaluated since variations in the quality of the Thiel embalmed human tissues was observed during the execution of this reperfusion project.

The purpose of this thesis was to install a prolonged dynamic 'lifelike' flow from artery to vein in Thiel embalmed cadavers. The following research questions were explored in consecutive studies that form the basis of this thesis:

1. What is the evidence for laparoscopic skills training on surgical training models? **(Part A)**
2. What types of reperfused human cadaver models used for surgical training are currently available and what is their evidence to learn surgical skills? **(Part B)**
3. Is it feasible to establish a dynamic flow in the vessels of fresh pig tissue? **(Part C)**
4. How does Thiel embalming solution spread in pig kidneys and how does long dynamic reperfusion with PP affect Thiel embalmed pig kidneys? **(Part D)**
5. What is the effect of ideal Thiel embalming circumstances on prolonged dynamic (micro)vascular reperfusion in a pig model and is it suitable for surgical practice? **(Part E)**
6. How does long dynamic reperfusion impact on Thiel embalmed human tissues? **(Part F)**
7. Are arterially pressurized Thiel embalmed cadavers useful for endovascular surgical training? **(Part G)**

PART A: Systematic review of laparoscopic skills training.

The tools for laparoscopic training include VT; live animal surgery; human and animal cadaver training; and VR.[98] The first objective of this thesis was to compare their effectiveness in learning laparoscopic skills with particular attention for the evidence of skills training on human cadavers. **Chapter 4** analyses the risk of bias of the included publications, summarizes the

characteristics of the individual studies (*i.e.* type of participants and type of interventions; training tasks; duration and frequency of training; and task and model used for skills assessment) and focuses on a qualitative synthesis of the outcome data (*i.e.* time to perform the task; error score; accuracy; composite score; and economy of movement).

PART B: Systematic review of reperfused human cadavers and body parts.

In 2001, Garrett et al. described the first human cadaveric circulation model in isolated sections of the arterial system and employed it for training vascular procedures.[97] Since then, simple to more complex reperfused models have been reported. The objective of **chapter 5** is to gain insight into the existing reperfused human cadaver models for surgical training, particularly in terms of characteristics of reperfused tissues; extensiveness of vascular flow; reperfusion techniques applied and their related advantages/limitations; surgical procedures trained; employment in training programs; skills assessment tools used; and skills transfer to patients.

PART C: Exploration and assessment of dynamic reperfusion in fresh pig tissues: two pilot studies.

Initial work on postmortem dynamic reperfusion has mainly focused on the establishment of optimal reperfusion conditions for high-resolution angiography of arteries and veins in fresh animal tissues and human extremities.[87, 88] Although a prolonged circulation was developed in fresh animal tissue, no reperfusion data such as flow rate, fluid loss and intravascular pressure were described.[87] **Chapter 6** is a feasibility study that describes our early experience with the installation of an ongoing flow from artery to vein in a fresh pig hindquarter using PP, with particular attention to reperfusion time, flow rate, intravascular pressure, leakage and edema. **Chapter 7** expands the findings of the previous study with specific focus on the distribution of PP in the vessels of fresh pig lungs and the amount and cause of perfusate leakage.

PART D: The spread of Thiel embalming solution in pig kidneys and subsequent long dynamic reperfusion of these embalmed organs.

Expanded knowledge of the tissue effects of Thiel embalming can facilitate long-lasting vascular reperfusion and permits to compare perfusates in terms of vascular spread, organ weight and volume increase and generated arterial pressures. **Chapter 8** describes the spread of a fixed Thiel embalming volume in pig kidneys. Reperfusion properties of PP and diluted PEG 400 were compared in these Thiel embalmed pig kidneys.

PART E: Long dynamic Thiel embalmed pig reperfusion, evaluation of the microscopic vessel recruitment and feasibility for surgical practice. A plea for pump-driven embalming.

Currently, at Ghent University Hospital, donated bodies are embalmed by gravity within 72 hours after death. This means that the embalming solution is lifted for several meters, creating

temporarily high infusion pressure and easier administration. Ideal circumstances for Thiel embalming with a pump as soon as possible after death may enhance embalming quality and subsequent vascular reperfusion. In **chapter 9**, pigs are embalmed with a pump immediately after euthanasia. A long reperfusion from artery to vein with PP was then assessed and its gross anatomical effects and feasibility for surgical training were observed. Moreover, the microvascular reperfusion in pump-embalmed pig small intestines was visualized and compared with that perceived in gravity-embalmed human tissues.

PART F: Preliminary results of long dynamic Thiel embalmed human tissue reperfusion.

Previous research has demonstrated the exceptional reperfusion properties of PP in pig tissue. In a next step, this was explored in human tissue prelevated from gravity-embalmed Thiel cadavers. **Chapter 10** mainly focuses on flow parameters and tissue effects during prolonged dynamic human kidney reperfusion at low and high flow rates. Preliminary results of one liver, one arm and one whole body reperfusion are added.

PART G: Arterially pressurized Thiel embalmed cadavers: an essential model for training.

In 2010, Grabherr et al. introduced a modified heart–lung machine that installs postmortem human cadaveric circulation to perform angiography and to diagnose vascular lesions that could explain the cause of death.[90] **Chapter 11** investigates if this technique allows testing endovascular procedures in Thiel embalmed human cadavers. Therefore, in contrast with the previously described dynamic reperfusion method, sole retrograde pressurization of the femoral artery and aorta was established and subsequent endovascular delivery of aortic valves was assessed radiographically and after extraction of the heart.

PART A

Systematic review of laparoscopic skills training

Chapter 4

Training models in laparoscopy: A systematic review comparing their effectiveness in learning surgical skills

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Abstract

Background Surgery has traditionally been learned on patients in the OR, which is time-consuming, can have an impact on the patient outcomes and is of variable effectiveness. As a result, surgical training models have been developed, which are compared in this systematic review.

Methods We searched PubMed, CENTRAL and Science Citation Index Expanded for randomized clinical trials and randomized cross-over studies, comparing laparoscopic training models. Studies comparing one model with no training were also included. The reference list of identified trials was searched for further relevant studies.

Results Fifty-eight trials evaluating several training forms and involving 1591 participants were included (four studies with a low risk of bias). VR or VT training versus no training improves surgical skills in the majority of trials. Both VR and VT are as effective in most studies. VR training is superior to traditional laparoscopic training in the OR. Outcome results for VR robotic simulations versus robot training show no clear difference in effectiveness for either model. Only one trial included human cadavers and observed better results versus VR for one out of four scores. Contrasting results are observed when robotic technology is compared with manual laparoscopy.

Conclusion VR and VT training are valid teaching models. Practicing on these models similarly improves surgical skills. A combination of both methods is recommended in a surgical curriculum. VR training is superior to unstructured traditional training in the OR. The reciprocal effectiveness of the other models to learn surgical skills has not yet been established.

Introduction

Abdominal surgery has traditionally been performed via an open approach. However, during the last decades, there is an obvious trend toward minimally invasive surgery worldwide making laparoscopic surgery the standard approach for several procedures. This irreversible change necessitates a dramatic adaptation of the current surgical curriculum. This adjustment is inevitable because performing minimally invasive surgical procedures often demands a high level of skills. Presently, the traditional teaching methods in the OR do not suffice to learn these skills because of the diminished training hours for surgical residents and the lack of time for surgeons to adequately teach techniques. Moreover, the long learning curve for most laparoscopic procedures together with the risk of serious complications when performed by inexperienced hands impede a fast learning of minimally invasive techniques.

As a consequence, surgical training models have been developed to serve as an adjunct to standard teaching in the OR. The most used laparoscopic simulation models are VR, VT, animal training and human cadaver training. Strikingly, together with the growing use of robots in surgical practice, robot technology has recently been applied in these training methods, apart from the manual laparoscopic equipment.

Numerous studies and several reviews evaluating the value of the previously described models reported their favorable training effects.[99-106] In addition, transfer of skills toward the OR, which is the purpose of training, has been shown in many reports.[98, 101, 102, 107, 108] Moreover, comparative trials have been developed to determine the real place of each model in a surgical training curriculum. In brief, the methodology, the participants, the training tasks, the assessment tools and the measured outcomes in these comparative trials are highly variable. As a result, a Cochrane systematic review about VR training for surgical trainees in laparoscopic surgery has stated that further research toward better methodological quality and more patient-relevant outcomes are needed. Despite the low quality of most included studies, this systematic review has concluded that VR training can supplement standard laparoscopic surgical training of apprenticeship and is at least as effective as VT.[109]

The aim of this review is twofold. Firstly, to extend the current knowledge of most of the outcomes as described in a Cochrane systematic review by adding the most recent literature and by using more inclusion criteria.[109] Secondly, as teaching surgical skills cannot be the only purpose of training, this review also intends to evaluate the value of human cadavers in learning surgical anatomy.

Materials and methods

A structured approach for this review was used as described in the Cochrane Handbook for Systematic Reviews of Interventions (Version 5.0.1).[110] Methods of the analysis and inclusion criteria were specified in advance and documented in a protocol which was not published.

Criteria for considering studies for this review

Types of studies

Only randomized controlled trials and randomized cross-over trials were considered for inclusion. Quasi-randomized studies, where the allocation sequence was not randomly generated, were excluded. The language of the trials and the year of publication were no exclusion criteria. Unpublished studies were not included.

Types of participants

Studies including medical students and surgical residents irrespective of their surgical experience as well as certified surgeons were eligible for inclusion.

Types of interventions

Trials comparing any surgical training model as VR training, VT training (using conventional laparoscopic equipment as well as robots), animal models and human cadavers were considered for inclusion. Also, studies comparing any of these methods versus no training or combinations of training models were included. Trials comprising comparisons with mirror box trainers or cardboard box trainers were also included in this review but comparison with these models was not performed.

Types of outcome measures

- Primary outcomes :

Time taken to perform the task.
Error score as defined by the authors.

- Secondary outcomes :

Accuracy as defined by the authors.
Composite score as defined by the authors.
Economy of movement as defined by the authors.

Studies were included if they compared any of these specific outcomes.

Search methods for identification of studies

Electronic searches

Studies were identified by searching electronic databases. The full search strategy for MEDLINE (through PubMed) is displayed in table 1. From January 2012 till April 2012, one author searched for randomized controlled trials and randomized cross-over trials using CENTRAL in The Cochrane Library 2012, Issue 3; MEDLINE (1966 to the present through PubMed); and Science Citation Index Expanded (1900 to the present). A last search was run on 13 April 2012. The search strategy was developed and conducted by WW.

Table 1. *Search strategy MEDLINE (through PubMed)*

#1	randomized controlled trial [Publication Type]
#2	controlled clinical trial [Publication Type]
#3	randomized [Title/Abstract]
#4	placebo [Title/Abstract]
#5	clinical trials as topic [mesh: noexp]
#6	randomly [Title/Abstract]
#7	trial [Title]
#8	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7
#9	animals [mh] NOT humans [mh]
#10	#8 NOT #9
#11	#10 AND train* [Title/Abstract]
#12	#10 AND surg* [Title/Abstract]
#13	#10 AND laparoscop* [Title/Abstract]
#14	#10 AND celioscop* [Title/Abstract]
#15	#10 AND peritoneoscop* [Title/Abstract]
#16	#10 AND virtual realit* [Title/Abstract]
#17	#10 AND box [Title/Abstract]
#18	#10 AND video trainer [Title/Abstract]
#19	#10 AND animal [Title/Abstract]
#20	#10 AND pig [Title/Abstract]
#21	#10 AND sheep [Title/Abstract]
#22	#10 AND rabbit [Title/Abstract]
#23	#10 AND inanimate [Title/Abstract]
#24	#10 AND human [Title/Abstract]
#25	#10 AND cadaver [Title/Abstract]
#26	#10 AND bod* [Title/Abstract]
#27	#10 AND corpse* [Title/Abstract]
#28	#10 AND simulat*[Title/Abstract]
#29	#10 AND MIST-VR[Title/Abstract]
#30	#10 AND lapsim [Title/Abstract]
#31	#10 AND lap man [Title/Abstract]
#32	#10 AND robot* [Title/Abstract]
#33	#10 AND bench model [Title/Abstract]
#34	#10 AND computer assisted [Title/Abstract]
#35	#10 AND education [Title/Abstract]
#36	#10 AND skills [Title/Abstract]
#37	#10 AND sutur* [Title/Abstract]
#38	#10 AND knot tying [Title/Abstract]
#39	#10 AND thiel [Title/Abstract]

Searching other resources

The reference list of identified trials was searched for further relevant studies.

Data collection and analysis

Selection of studies

Each stage of the study selection process was performed by the same researcher (WW) who was not blinded to the names of the study authors, their institutions, the journal of publication or the results. Firstly, the titles and abstracts of the retrieved records were screened. Then, the full-text report of all possibly relevant abstracts were searched and examined whether they met the inclusion criteria. Special attention during the search was given to detect duplicate publications. If no full report was available, the original investigators were contacted.

Data extraction and management

A data collection form was made as described in the Cochrane Handbook for Systematic Reviews of Interventions.[111] This form was pilot-tested on five randomly-selected included studies and refined accordingly. Finally, only one form was used. One author (WW) extracted the pre-specified data as described below:

1. Year of publication
2. Setting and country
3. Inclusion criteria
4. Number of participants
5. Type of training models
6. Evaluated tasks in each group
7. Duration of training in each group
8. Assessment tool
9. Outcomes (described above)
10. Methodological assessment (described below)

Only those outcomes of interest in the review were collected. In addition, key conclusions and comments by the study authors and review authors were noted. No variables were added to the data collection form after the review started.

Assessment of risk of bias in included studies

All studies that met the selection criteria were individually assessed for methodological quality in an unblinded manner at both study and outcome level. Therefore, a component approach based on domains was used. One author assessed the domains of sequence generation, allocation concealment and blinding of the outcome assessors. Moreover, incomplete outcome and selective outcome reporting and whether selection bias occurred were evaluated. For each

question-based item, the author described what was reported to have happened in the study. Then, a judgment (Yes for low risk of bias, No for high risk of bias or unclear) relating to the risk of bias was made. Trials with adequate methodological quality in all the six domains were considered to be trials of low-bias risk.

Measures of treatment effect

Although a meta-analysis was not made, the intended summary effect measure for each type of outcome is described below:

1. Dichotomous variables: the risk ratio and odds ratio.
2. Continuous variables: mean difference and standardized mean difference.

Subgroup analysis and sensitivity analysis were not performed.

Results

Description of studies

Results of the search

Figure 1 illustrates the flow of studies through this review. Using MEDLINE, CENTRAL and Science Citation Index Expanded 80 studies were identified. Scanning reference lists of these randomized trials revealed three extra studies. These 83 trials were retrieved for further assessment. Nineteen studies were excluded as they did not meet the inclusion criteria. Moreover, it was not possible to retrieve the full report of six trials despite contacting the study authors. A total of 58 trials (57 references) were finally selected for inclusion.

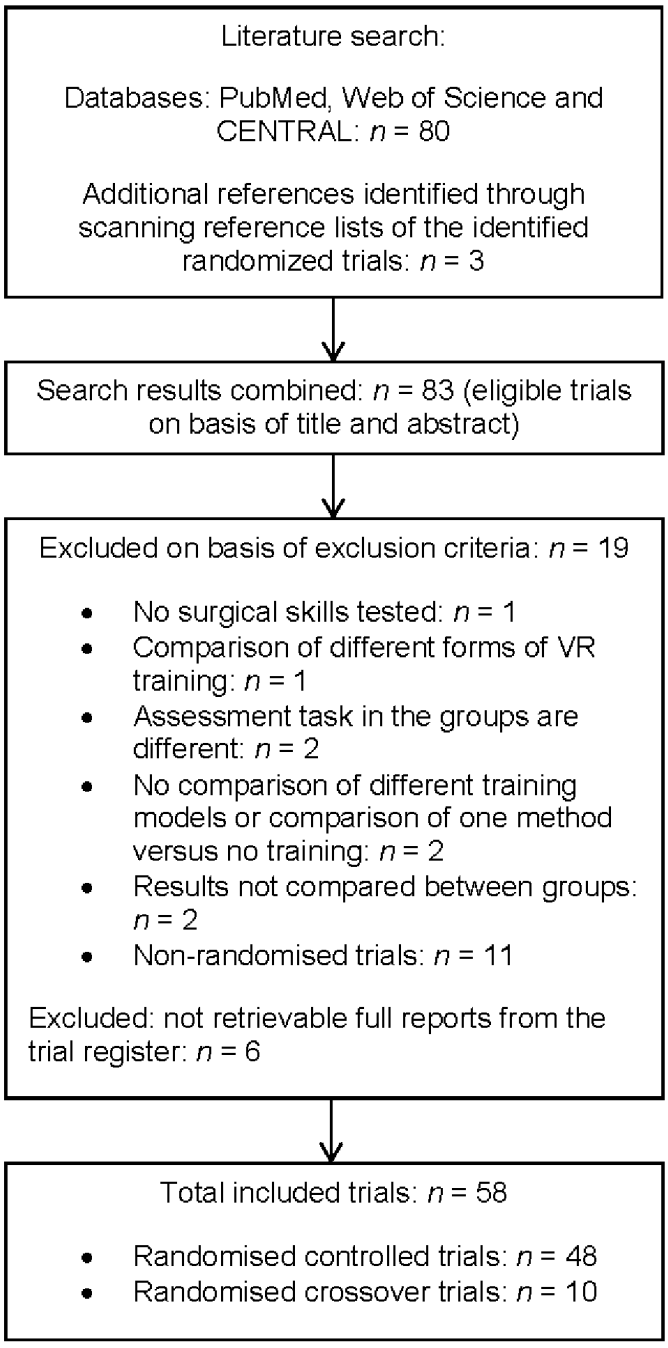

Figure 1. *Flow diagram which summarizes the study selection processes*

Characteristics of the included studies

Fifty-eight trials involving 1591 participants were identified. Forty-eight studies were randomized controlled trials, whereas ten studies were randomized cross-over trials. These trials were reported in 56 full papers. Two separate studies were reported in one full paper and one study was published as an abstract in the supplements of a journal.[112, 113] One duplicate was identified, however, using different assessment models to evaluate effectiveness of training.[114, 115] Therefore, both studies were considered as unique trials. Forty-one trials compared training versus no activity; 23 trials compared VR versus another training form (VT training (*n* = 15), standard laparoscopic training in the OR (*n* = 3), robot training (*n* = 3) and human cadaver training (n = 1); and VR and VT versus VT (*n* = 1); six studies compared robot training versus VT training (*n* = 5) or laparoscopic animal training (*n* = 1); one study described laparoscopic animal training combined with VT versus VT.

The characteristics of the studies are summarized in the 'Characteristics of included studies' table **(Appendix 1)**. We did not contact the authors to obtain individual participant data. Every included study was published in English and the majority was performed in the USA (31 out of 58). Eighteen studies assessed the training tasks on animal tissue whereas 14 trials evaluated training skills on patients. Only one trial used fresh human cadavers to train laparoscopic sigmoidectomy.

Risk of bias in included studies

An extensive evaluation of each study on each criterion based on the Cochrane Collaboration's tool for assessing the risk of bias is reported in a 'Risk of bias summary' **(Appendix 2)**. Only four studies were considered to be of low risk as the six domains were positively assessed.[99-102] None of the other 54 trials reported both adequate sequence generation and allocation concealment. Blinding of outcome assessors was reported in 35 trials.

Effects of interventions

Outcome data from the individual studies are presented in the 'data and analysis' table **(Appendix 3)**. "Because the methodological quality of the studies, the participants, interventions, assessment tools, reported outcomes varied markedly, the focus of this review was describing the studies, their results, their applicability and their limitations and on qualitative synthesis rather than meta-analysis".[116]

Several definitions were used for each outcome across the included studies:

1) Primary outcomes:
   a. Time: taken to complete the task, reduction in time (*e.g.* percentage) to perform the task (post-training versus pre-training status), speed in performing the task or change in speed after comparison with pre-training status.

b. Error score: tissue handling, composite error score, number of errors (dropping object, perforation of the object), complications and hospital stay.

2) Secondary outcomes:

a. Accuracy: excess needle manipulations, mean dot scores for suturing tasks and number of damaged beads.

b. Composite score: several forms used in the included studies.

c. Economy of movement: path length of instruments, speed of instruments, total hand movements and efficiency.

Training versus no training

1. VR training versus no training

This review included 20 studies, which analyzed this comparison.[8, 10, 98-100, 113-115, 117-127] Seven[99, 100, 113, 114, 117, 119, 124] and five trials[8, 10, 113, 118, 121] assessed the effect of training using pig tissue or patients, respectively.

a. *Time*

Time was evaluated in 16 studies.[8, 98-100, 114, 115, 118-127] Half of these trials showed statistically significant better time scores following training.[8, 99, 115, 120, 122-124] Moreover, a statistically significant difference was found in an additional trial for five out of six evaluated tasks.[119] In contrast, six studies observed statistically non-significant difference for this outcome.[98, 114, 118, 121, 125, 126] The same finding was also noted in one study for 3 out of 4 analyzed tasks.[100]

b. *Error score*

The error score was assessed in 11 studies, of which five trials[8, 115, 118, 120, 121] mentioned a statistically significant better result in case of VR. This was confirmed as well in one extra study if an objective scoring system was used. However, if evaluation was done with a subjective score a statistically non-significant difference was obtained in this trial.[124] Five studies did not demonstrate any statistically significant difference concerning this outcome.[98, 100, 113, 114]

c. *Composite score*

This scoring system was measured in seven studies. VR significantly outperformed no activity in five studies.[10, 99, 114, 115, 119] A statistically non-significant difference was found in one trial[117] and for three out of four tasks in a second study.[100]

d. *Economy of movement*

Three trials reported a statistically significant better result for this outcome after training.[8, 99, 120] A fourth study showed statistically significant difference in number of movements taken by each hand (increase for the non-dominant left hand and decrease for the right hand).[126] A statistically non-significant difference was observed in four studies.[98, 113, 124]

2. VT training versus no training

Nineteen trials analyzed this comparison.[14, 98, 100, 123, 125-138], of which 6 assessed training effect on pig tissue[100, 129, 133, 137-139] and four on patients.[128, 131, 134, 135]

a. *Time*

Fourteen studies described this outcome.[98, 100, 123, 125-131] Half of these trials showed statistically significant difference (shorter time, greater time improvements and better time scores) when subjects trained on the VT.[123, 127, 129-131] This was also noted in another trial for two out of four tasks.[100] A statistically non-significant difference was reported in four trials[98, 125, 126, 137], whereas one study noted statistically non-significant difference in the time post-training, but a statistically significant difference in tie time difference between baseline and post-training.[132] Similarly, statistically significant larger median time reductions pre-training versus post-training were measured in an additional trial using VT training, whereas a statistically non-significant difference between both groups was found when tested on the porcine model.[134]

b. *Error score*

Although a non-significant difference was calculated in one study[100], VT training resulted in statistically significant better scores in the majority of trials.[98, 128, 130, 134, 135]

c. *Composite score*

Eight out of 11 studies reported statistically significant higher composite scores in the training group.[14, 128, 133-136, 138, 139] A statistically non-significant difference was found in two trials[131, 137] and for three out of four tasks in a third study.[100]

d. *Economy of movement*

Statistically significant better economy of movement scores were encountered in two trials following VT.[98, 130] One paper reported a significant reduction in the number of movements of the dominant (right) hand, however, a significant increase in the number of movements of the left hand was noted.[126] Similar results for both compared groups were reported in two studies.[135, 137]

3. VR training and VT training versus no training

Two studies analyzed the training effect on either patients or pigs.[100, 140]

a. *Time*

Similar results were found for both studies. Gastrogastric knot tying in a laparoscopic Nissen model was done in a statistically significant shorter time (sec) (mean ± Standard Deviation (SD)) (525.6 ± 189.6 vs 789.5 ± 171.3) ($P < 0.003$) for the trained group.[140] Likewise, three tasks (placing a piece of bowel in a bag, liver biopsy and 'running the bowel') in a porcine model were performed faster after training ($P < 0.02$), except for placing a stapler on the bowel (no *P*-value).[100]

b. *Error score*

Statistically significant fewer errors (mean ± SD) (25.6 ± 9.3 vs 37.1 ± 10.2) ($P < 0.01$) were found in the trained group while performing two gastrogastric knots in a patient.[140] The study using the pig noted similar numbers of errors for all tasks between both groups (no *P*-value).[100]

c. Accuracy

Statistically significant (mean ± SD) (18.5 ± 10.5 vs 27.3 ± 8.5) ($P < 0.05$) fewer excess needle manipulations were described after training in one trial.[140]

d. *Composite score*

An overall subjective score by a blinded assessor revealed no statistically significant difference for all groups (no *P*-value) except for one out of four tasks ('running' the bowel) ($P < 0.03$).[100]

VR training versus other training forms

1. VR training versus VT training

Fifteen studies compared both training models[98, 100, 107, 108, 123, 125-127, 141-147], of which four assessed the effectiveness of training on pig tissue[100, 142, 144, 147] and one on patients.[107]

a.Time

Thirteen trials evaluated this outcome. Eight trials noted statistically non-significant difference among both groups.[98, 100, 126, 127, 144, 145, 147, 148] This was also the case in a cross-over study for two out of three tasks. However, a statistically significant faster task performance in the VT-VR group versus the VR-VT group was noted for the third task (elastic band) when performed using the VT trainer.[141] The VT group performed tasks statistically significant shorter in two trials which were assessed on this VT model.[107, 123] In contrast, one trial noted a statistically non-significant difference between both groups when tested on the VT, while tested on the VR a statistically significant shorter time was reported for the VT group.[108] One cross-over trial found for two out of three tasks (cutting and knot-tying) a statistically significant superior performance achieved during training on one simulator to that achieved on the same simulator by the other group.[146]

b. Error score[98, 100, 143, 146, 147]

One out of five trials noted a statistically significant better score (P = 0.0008) after VR training, while the other studies did not mention any statistically significant difference between both groups following training.[143]

c. *Composite score*[100, 107, 108, 142, 144, 147]

Four out of six trials evaluated both training models in a pig model[100, 142, 144, 147], whereas one study evaluated a subgroup on patients.[107] Using the composite score, a statistically non-significant difference was found for three trials and for three out of four tasks in a fourth trial.[100, 142, 144, 147] In contrast, subjects who trained on the VR trainer performed statistically significant better when objectively scored on the VR trainer in two trials.[107, 108] Notably, one of these trials used a second scoring system (subjective) to do a subgroup analysis in postgraduate year (PGY) two surgical residents who trained on patients (CCE), which showed statistically non-significant difference between both training models.[107]

d. *Economy of movement*[98, 108, 126, 141, 146]

A statistically non-significant difference was found for two out of five trials[98, 126] and for two out of three tasks in an additional cross-over trial[141]. The latter trial evaluated both groups post-training using both models and noted that the VT-VR group significantly outperformed the VR-VT group for both hands when tested on the VT and for the right hand when tested on the VR. One trial reported statistically significant superior plateau performance in the VR group versus the VT group for all three tasks.[146] This was also the case for another study when the post-training was assessed on the VR trainer. However, when tested on the VT trainer there was a statistically non-significant difference.[108]

2. VR training versus standard laparoscopic training in the OR

Three trials were identified, evaluating the training effect on patients.[101, 102, 112]

a. *Time*

VR training resulted in a statistically significant shorter operation time (salpingectomy, CCE and total extraperitoneal (TEP) hernia repair).[101, 102, 112]

b. *Error score*

Statistically significant fewer errors (11.7 vs 19.7) (P < 0.01) were made during laparoscopic CCE in one trial following VR training.[112] One study evaluated patient-related outcomes and found statistically significant fewer intraoperative and postoperative complications (of any type) and overnight stay (all P < 0.05) after VR training.[102]

c. *Composite score*

There was a statistically significant better operative performance in two trials following VR training.[101, 102]

3. VR training versus robot training

Three trials were included.[148-150] One study evaluated the training effect on a porcine model.[150]

a. *Time*

This outcome was evaluated in two studies. One paper compared two exercises. A pick-and-place task (bimanual carrying) showed a statistically non-significant difference ($P > 0.05$). The second task (passing a needle through holes) revealed a statistically significant shorter time for VR ($P = 0.003$).[148] The other study compared the movement of rings through a wire, which was statistically non-significant different for both training models ($P = 0.21$).[149]

b. *Composite score*

Both trials reported a statistically non-significant difference in improvement of scores from the pre-training status to the post-training status ($P > 0.05$).[149, 150]

c. *Economy of movement*

Total travelling distance of the instrument tip during the pick-up-and-place task (bimanual carrying) was comparable for both groups ($P > 0.05$).[148] However, during the needle passing task a statistically significant shorter distance was noted for the VR group ($P < 0.001$).

4. VR training versus fresh human cadaver training

This trial compared the laparoscopic sigmoidectomy procedure.[104]

a. *Composite score*

Four scores were evaluated for both trainers and trainees. Generic events (appropriate incisions, planes of dissection, instruments and diathermy use, retraction, hand-eye coordination, tissues handling, suturing technique, bleeding control and speed of procedure) were statistically significant better (= lower) scored on the cadaver for trainers (mean ± SD) 1.0 ± 0.1 vs 1.1 ± 0.3 ($P = 0.020$) as well as for trainees 1.0 ± 0.1 vs 1.1 ± 0.2 ($P = 0.034$). However, statistically non-significant differences were noted for the other scores (specific and generic skills and specific events) ($P > 0.05$).

5. VR training and VT training versus VT training

This review included one trial comparing these interventions.[151]

a. *Time*

Statistically non-significant difference ($P > 0.05$) was reported among both groups to complete the tasks.

b. *Error score*

Statistically non-significant differences ($P > 0.05$) were found among both training groups.

VT training versus robot training

Five trials were included, of which one[152] evaluated the training effect on pig tissue.[152-156]

a. *Time*

Time to complete tasks differed among the studies. VT training outperformed robot training in two trials (three out of four tasks[154] and two out of five tasks[152], respectively). This difference was statistically significant. In contrast, one study noted that performances were statistically significant faster for three out of five tasks using robotics.[155]

b. *Error score*

One study observed fewer errors in the robot group[153], but statistically non-significant difference was found between both groups for the majority of tasks (4/5) in another publication (no *P*-value)[152]. However, the VT groups made statistically significant more errors for rope-passing (median number (range) (13.0 (3-31)) vs 6.0 (1-24)) ($P = 0.05$).[152]

c. *Accuracy*

Statistically non-significant difference for suturing and knot tying was noted in two trials[154, 155], but statistically significant better accuracy was found for the robot in two out of four tasks in one trial.[155]

d. *Composite score*

Subjects who trained on the VT had statistically significant higher scores (mean ± SD) (84 ± 75 vs 56 ± 63) ($P < 0.001$) for knot tying.[157] In contrast, one study revealed that robot training resulted in a better performance score (no *P*-value).[153]

Laparoscopic animal training versus other

1. Laparoscopic animal training versus robot animal training

One trial reported this comparison in a porcine model.[103]

a. *Time*

Robot training resulted in a statistically significant faster performance of tasks (600 (473–600) vs 460 (143–600) sec) ($P < 0.001$).

b. *Error score*

Statistically significant fewer errors (1 (0–6) versus 0 (0–2)) ($P < 0.001$) were made in the robot group.

c. *Composite score*

Subjects using the robot had statistically significant higher suturing scores (0 (0–8) versus 0 (0–457) ($P < 0.001$).

2. Laparoscopic animal training and VT training versus VT training

One trial reported this comparison.[157]

a. *Time*

Adding laparoscopic animal training to VT resulted in a statistically significant difference in percentage time improvement (mean ± SD) (34.3 ± 5.7 vs 7.3 ± 9.2) ($P = 0.0001$: 95 % CI) compared with VT alone.

Discussion

The purpose of this review was to extend the current knowledge reported in a Cochrane systematic review using their predefined outcomes.[109] Therefore, the most recent literature (2009 to the present) as well as a wide range of comparisons of interventions were added. In detail, trials analyzing VT training versus no training or robot training; VR training versus robot training or human cadaver training; and laparoscopic animal training versus robotic animal training were identified. The main findings of this systematic review are discussed below.

Estimating the actual value of a training model itself can be realized by comparing with a non-trained group. As a result, qualitative synthesis of training with a VT shows its effectiveness in slightly more than half of the trials in terms of time. Remarkably, almost every trial reports better error and composite scores after VT training. Evidence for a trend toward better economic performance after VT training is noted in a smaller amount of trials. Equal conclusions can be made for VR training. Again, a similar proportion of trials demonstrate a superior reduction in time to complete tasks following exercising in a virtual environment. This is also the case for error scoring, confirming better composite scores after VR training in almost every trial. The included trials observe in half of studies improved economic movement result too. A meta-analysis for time outcomes (time taken to do the job) performed in a Cochrane review confirms our findings as the VR group performed the tasks more quickly. However, the reduction in time between first (before) and second (after training) assessments, difference in the speed or in the improvement of speed between the two groups was not statistically significant in this meta-analysis.[109] Note that in the current review this breakdown of time as an outcome in several definitions has not been done. The other outcomes were not

put into meta-analysis in the Cochrane review and their results were not as convincing as time evaluation. Our analysis of these outcomes, based on more included studies, more strongly confirms the significant effectiveness of VR training.

Interestingly, a comparison of a combination of both VR and VT models versus no training has been done in two trials. Not unsurprisingly, positive training effects are noticed. However, robust conclusion about the advantage over training using a single model cannot be made.

Equally interesting is the comparison of VR training versus VT training. Most included studies notice no significant difference for time among both training groups. This is confirmed in the meta-analytical review.[109] Although one recently published study reports significantly shorter times for knot tying on a rubber sheet, this assessment has been done in a VT model. Moreover, this study probably has considerable bias.[141] This review finds no difference for error scores between both VT and VR groups for four out of five included trials. This finding is in accordance with the Cochrane analysis.[109] When composite scores are evaluated, slightly more than half of the trials show no difference among both groups. However, VR training was superior according to two trials, but this assessment was done on the VR simulator.
In general, all trials that compare VR training with standard laparoscopic training in the OR describe superior performance after VR training. This is in accordance with the literature and confirms that a structured teaching is more effective than traditional training.[109]

No clear conclusions can be made when validating VR versus robot training. Only three trials are included. The results for time and economy of movement vary. More consistency has been found for the composite score, which revealed no significant difference for two trials.

Only one study evaluates fresh human cadavers. This comparative trial shows that training a complex surgical procedure as a laparoscopic sigmoidectomy on a fresh human cadaver results in improved dissection, retraction, hand-eye coordination, suturing, bleeding control and speed of procedure compared with VR simulation. Note that this superiority is only significant for one out of four evaluated scores. Moreover, no extra assessment tool, *e.g.* in the OR on a patient was used in this study to further analyze the value of fresh human cadavers. Although the results are promising, better comparative trials on human cadavers are necessary. These trials could also contribute in learning surgical anatomy.

In recent years, robotic technology has been introduced in the surgical world. Its value has been mostly compared with manual laparoscopy in terms of time to perform tasks. However, further studies are needed as contrasting time results have been published. These findings differ from one study which evaluated the same techniques in a porcine model showing better performance for time, errors and composite score in the robot model.

Importantly, despite a high number of included studies in this systematic review a considerable amount of comparisons are based on a few studies. As only four of the included trials were free of bias, strong conclusions cannot be made except for VR or VT versus no activity and for VR versus VT or standard laparoscopic training in the OR.

Although only one study assessed training on human cadavers, it is surely positive that a huge amount of included studies evaluated training effect on pig tissue and patients, indicating a trend toward evaluating transferability of obtained skills.

This systematic review has also some notable limitations. Only one author searched for trials, evaluated their eligibility, extracted data and assessed the methodological quality.

In conclusion, VR training and VT training are valid teaching tools. Exercising on these models equally improves surgical skills. A combination of both methods is recommended in a surgical curriculum. VR training is superior to unstructured traditional training in the OR. The reciprocal effectiveness of the other models to learn surgical skills has not yet been stated.

PART B

Systematic review of reperfused human cadavers and body parts

Chapter 5

Systematic review of surgical training on reperfused human cadavers

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To be submitted

Abstract

Background The role of reperfused human cadavers in surgical training has not been established. This review analysed the literature on reperfused human cadaver models and their educational applications in surgery.

Methods MEDLINE and Science Citation Index Expanded were used to identify reports describing reperfused human cadaver models in terms of simulated surgeries, the use of tools to assess technical competency and skills transfer to patients, cadaver status (fresh/embalmed) and vascular reperfusion techniques.

Results Twenty-seven reports were included in the review. Most participants practised vascular surgeries (*n* = 23), flap techniques (*n* = 4), or trauma procedures (*n* = 2). Training progression was evaluated objectively in one study, but no data have been published. In two studies, new flap techniques were practised on cadavers and repeated successfully in patients. Whole and partial bodies were equally employed (*n* = 12). Thirteen studies each used fresh bodies and embalmed cadavers. Most embalmed cadavers were formaldehyde fixed (*n* = 9), resulting in stiffness. Few trainings were offered on soft Thiel-embalmed cadavers (*n* = 3). Only arteries were reperfused in 16 studies, while in 10 studies, the arteries and veins were filled. Arteries and/or veins were mostly pressurized (*n* = 16) and arterial flow was generated in 11 studies. No lifelike artery–vein flow was established in complete cadavers.

Conclusion Various reperfused human cadaver models exist, enabling practise of mainly vascular procedures. Preservation method determines the level of simulation fidelity. Thorough evaluation of these models as surgical training tools and transfer effectiveness is still lacking.

Introduction

The anatomical dissection of human cadavers has been used traditionally for teaching and study purposes.[158] Recent research has focused on the use of reperfused human cadavers to assess the vascular system for forensic diagnostic purposes.[83] For instance, in 2010, Grabherr *et al*. established circulation in cadavers with a modified heart-lung machine to detect the cause of death.[90] This technique has become a standard part of autopsy procedures.[90, 159] Reperfused human cadaver models are also suitable for the refinement of contrast injection dosages for CT examinations and for the testing of new devices.[160-162]

Post-mortem reperfusion creates new opportunities for surgical training; in particular, it allows surgical residents to learn advanced and/or new minimally invasive techniques without compromising patient safety. In 1993, Szinicz *et al*. developed a pulsatile porcine organ perfusion model for the practising of laparoscopic procedures.[86] Although this model has merits, non-human anatomy makes it less user-friendly.

Numerous reviews of surgical training models[5, 106, 163-165] and post-mortem reperfusion techniques in forensic medicine[82, 83, 166] have been conducted, but no systematic review of the role of reperfused human cadaver models in surgical training has been published. The aim of this review was to analyze the literature on reperfused human cadaver models and their educational applications in surgery. A systematic search was performed to identify studies using partial and complete human bodies, in which arteries and/or veins were reperfused to simulate lifelike circulation. The intent was to compare surgical procedures tested; applications in surgical training programmes; cadaver status (fresh *vs*. embalmed); vascular reperfusion technique and extensiveness; and advantages and limitations of the models.

Materials and methods

Inclusion and exclusion criteria

Publications, including abstracts and supplements, describing studies of human cadaveric reperfusion in surgical training were considered for inclusion in this review. Only studies published in English were included, independently of the year of publication.

Studies considered for inclusion in the review involved medical students, surgical residents and board-certified surgeons, regardless of the level of surgical experience. Only post-mortem studies in which flow or vessel pressurisation was established in whole human cadavers and body parts to practise surgical interventions were considered. Trials testing new devices and non-human, anatomical and imaging studies were not included in this review.

Outcome measures

The primary outcome examined was surgical procedures performed. Secondary outcomes were applications in surgical training programmes in terms of objective assessment of skills gained and transfer of skills gained to patients; human cadaver status (fresh *vs.* embalmed); vascular reperfusion technique; and advantages and limitations of the reperfused models, as described by the study authors. Studies not assessing or reporting on specific outcomes were not excluded.

Search strategy

The search methodology for this review was based on Chapter 6 of the Cochrane Handbook for Systematic Reviews of Interventions (version 5.0.1).[167] In January 2015, two authors (WW and FT) independently searched the MEDLINE (1966–present, *via* PubMed) and Science Citation Index Expanded (1955–present, *via* Web of Science) databases. The last search was conducted on 20 January 2015. A wide range of free-text terms and the following medical subject heading (MeSH) terms were used in various combinations: 'anatomy', 'angiography', 'bleeding', 'cadaver*', 'corpse', 'dissection', 'education', 'embalm*', 'endoscop*', 'experimental model', 'extracorporeal circulation', 'formalin', 'laparoscop*', 'live surg*', 'microsurgery', 'paraffin', 'perfusion', 'polyethylene glycol', 'postmortem', 'pressur*', 'research', 'surgical training' and 'teaching'. When a search resulted in the retrieval of more than 300 records, the results were discarded and the search was refined. The full search strategy for PubMed is illustrated in **Appendix 4**. The reference lists of identified articles were reviewed manually to identify additional relevant studies.

Study selection

The assessment of study eligibility and data extraction were based on Chapter 7 of the Cochrane Handbook for Systematic Reviews of Interventions (version 5.0.1).[111] Two authors (WW and FT) independently screened titles and abstracts of the retrieved records. The reviewers were not blinded to the names or affiliations of study authors, the journals of publication, or the study results. Full texts and abstracts of articles identified as potentially relevant were then examined to determine whether they met the inclusion criteria. Special attention was given to detect duplicate publications. When no full report was available, the authors were contacted. Disagreements between reviewers were resolved by consensus.

Data extraction and management

Two authors (WW and FT) independently extracted pre-specified data from publications using a data collection form designed for this study. The form had been pilot tested and revised (addition of variables) accordingly. The following data were extracted: surgical procedures practised; use of objective tools to assess skills; organisation of regular workshops on

reperfused models; type(s) of participant; skills transfer to patients; use of complete and/or partial human cadaver(s); number of reperfused bodies/body parts; cadaver status (fresh/embalmed, embalming technique); reperfusion of arteries and/or veins; type of perfusate used (including heated/non-heated); reperfusion technique; flow and pulsations established; and advantages and limitations of the reperfused model. Key conclusions and comments reported by study and review authors were also noted. Missing information was obtained by contacting the original investigators and asking open-ended questions. We contacted 11 authors for further information. Eight responded and provided the necessary information. Risk of bias and measurement of treatment effect were not assessed, as the studies included in this review were descriptive and did not involve the objective evaluation of training progress using validated methods.

Results

Screening of 163 publications identified as potentially relevant based on titles and abstracts led to the assessment of 30 publications to determine eligibility for study inclusion. Eight of these publications were excluded because they did not meet the inclusion criteria. Five additional studies identified from the reference lists of identified articles were included in the study sample. Thus, the final sample comprised 27 reports on descriptive studies, including two journal supplements. No studies were reported in more than one publication. No disagreement between reviewers concerning study inclusion occurred. Figure 1 illustrates the flow of study selection.

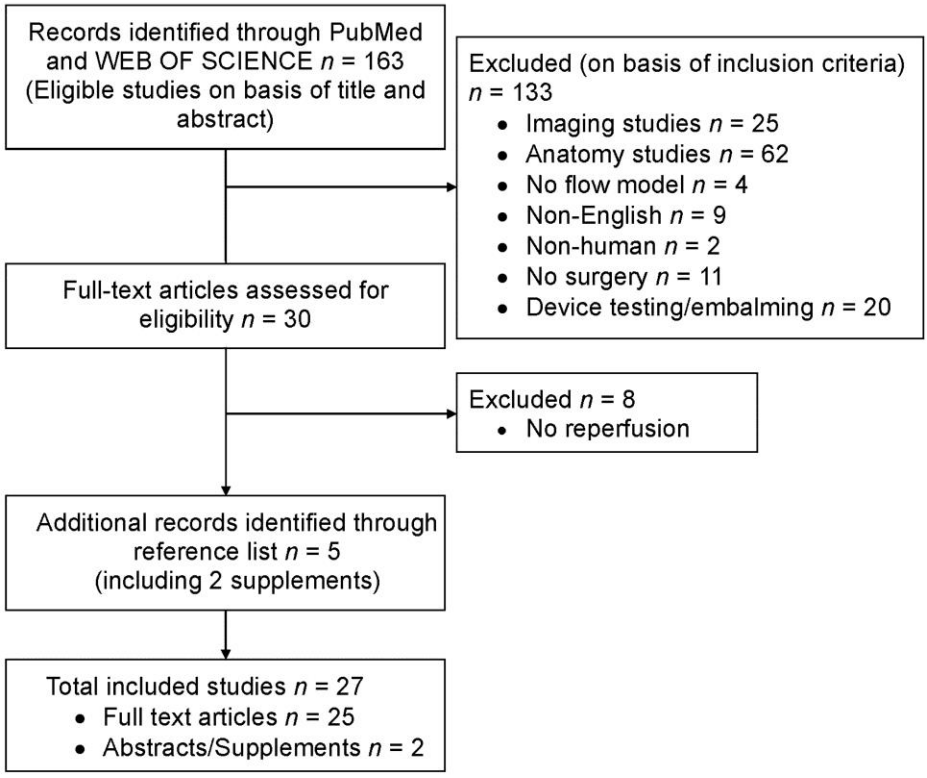

Figure 1. *Flow chart of study selection.*

Primary outcome: surgical procedures

The main surgical specialties for which human cadaver model were used were neurosurgery (*n* = 10)[92, 93, 168-175], vascular surgery (*n* = 9)[91, 97, 176-182], plastic surgery (*n* = 4)[29, 30, 59, 94] and cardiac surgery (*n* = 1)[28] (Table 1). Endovascular specialties, such as interventional radiology and cardiology, were only involved in a minority of studies.[91, 95, 96, 183] The target groups during training were board-certified specialists, surgical residents and scrub nurses. The following procedures were carried out: vascular dissection (*n* = 12)[28, 30, 92-95, 168-170, 173-175], vascular anastomosis (*n* = 10)[28, 93, 94, 96, 168-170, 172, 174, 175], endovascular procedures (*n* = 10)[91, 96, 97, 176-182], vascular bypass (*n* = 9)[28, 30, 96, 168-170, 173-175], flap procedures (*n* = 4)[29, 30, 59, 94], trauma surgeries (*n* = 2)[95, 96] and wound closure (*n* = 1)[94]. Some workshops offered exercises in haemostasis (*n* = 3)[92, 94, 170], aneurysm creation and/or repair (*n* = 3)[170, 175, 177], resection of artificial tumours (*n* = 2)[170, 171], endoscopic brain surgery (*n* = 1)[170], neck dissection (*n* = 1)[95] and craniotomy and cisternal dissection (*n* = 6)[93, 170, 172-175]. One publication did not specify the type of intervention.[183] In addition, nine studies involved the use of radiographic imaging[29, 91, 97, 173, 177, 178, 180-182] and one group infused indocyanine green (ICG) to evaluate graft perfusion[94].

Table 1. *Surgical techniques performed on reperfused human cadaver models. A, anastomosis; B, vascular bypass; C, wound closure; D, vascular dissection; E, endovascular surgery; F, flap surgery; n.r., not reported; SELANA: sutureless excimer laser assisted non-occlusive anastomosis; STAP, superficial temporal artery perforator flap.*

Study	Involved specialty	Type of surgery	New techniques tested?
Aboud *et al.*[170]	Neurosurgery	A, B, D, craniotomy, dura mater opening, sylvian fissure splitting, vessel clipping, artificial tumor resection and endoscopic intraventricular procedures	No
Aboud *et al.* [183]	Various specialties	n.r.	No
Aboud and Moursi.[96]	Various specialties	A, B, E and trauma surgery	No, but possible
Aboud *et al.*[95]	Trauma, vascular and general surgery	D and trauma surgery (stab wounds to the heart, lungs, hepatic lobe and vena cava; tracheostomy; cricothyroidotomy; open fractures; neck surgery on injured vessels)	No
Arbatli *et al.*[176]	Vascular surgery	E	No
Bouma *et al.*[28]	Cardiac surgery	A, B, D sternotomy	No
Canaud *et al.*[180]	Vascular surgery	E	No
Canaud *et al.*[181]	Vascular surgery	E	No
Carey *et al.*[94]	Plastic surgery	A, C, D and F	No
Chapter 11 of this thesis	Interventional radiology, vascular surgery and cardiology	E, sternotomy and surgical heart extraction	No, but possible
Faure *et al.*[182]	Vascular surgery	E	No
Garrett[97]	Vascular surgery	E	No, but possible
Gragnaniello *et al.*[171]	Neurosurgery	Tumor resection	No

Study	Involved specialty	Type of surgery	New techniques tested?
Güvencer *et al.*[172]	Neurosurgery	A and craniotomy	No
Jongkind *et al.*[177]	Vascular surgery	E and aneurysm repair	No
Kawashima *et al.*[168]	Neurosurgery	A, B and D	No
Kawashima *et al.*[169]	Neurosurgery	A, B and D	No
Linsen *et al.*[178]	Vascular surgery	E	No, but possible
Malikov *et al.*[30]	Plastic surgery	B, D and F	Bypass flap for distal revascularization
Numan *et al.*[179]	Vascular surgery	E	No
Olabe et al.[175]	Neurosurgery	A, B, D and clipping of aneurysms	No
Olabe *et al.*[173]	Neurosurgery	B, D and craniotomy	No
Pham *et al.*[92]	Neurosurgery	D and haemostasis of injured vessels	No
Russin *et al.*[93]	Neurosurgery	A, D and craniotomy	No
Scaglioni *et al.*[29]	Plastic surgery	F	STAP for ear reconstruction
van Doormaal *et al.*[174]	Neurosurgery	A, B and D	SELANA
Wolff *et al.*[59]	Plastic surgery	F	No

Secondary outcomes

Application in surgical training programmes

Seven studies were conducted in workshop settings.[28, 59, 92, 94, 95, 97, 171] Presently, none of these groups regularly organises workshops. Only Bouma *et al*. evaluated the skills required to safely perform beating heart coronary anastomoses during a 2-day training session on Thiel embalmed corpses.[28] Skills were assessed using a validated modified Objective Structured Assessment of Technical Skills (OSATS) published by Fann *et al.*[184] This OSATS decomposes the procedure (performing a coronary anastomosis) in 10 teachable components (*i.e.* arteriotomy, graft orientation, bite appropriate, spacing appropriate, use of needle holder, use of forceps, needle angles, needle transfer, suture management/tension and knot tying. Each component is scored from 1 to 5 (poor, below average, average, good and excellent). The modified OSATS contains two additional components (cardiac positioning and intracoronary shunt placement). Scores range between minimum 12 and maximum 60. The OSATS is first used in a formative way (*i.e.* to provide feedback on areas of strength and weakness) and finally in a summative way (*i.e.* to determine whether required competencies and standards have been reached). Residents are required to perform at least 10 anastomoses and score at least 48 points on four OSATS before they can ask for a final summative OSATS. They are required to score at least 48 points on the final summative OSATS ('good' on all components) before they can proceed to their training on patients. No data on skills transfer to real life were published.

No study assessed skills transfer to patients. Two studies designed and practiced an advanced flap technique on cadavers prior to carry it out on actual patients.[29, 30] Malikov et al. raised 32 bypass flaps in 16 preserved (embalming not specified) human corpses. This innovative technique of distal revascularisation based on the thoracodorsal arterial axis provides an arterial graft and a free flap supplied by a collateral branch of the graft.[30] Clinical applications of this flap were assessed in three patients with severe tissue loss following ischaemia of the inferior third of the leg (n = 2) and the foot (n = 1). No flap occlusion occurred and tissue defects healed, proving the feasibility and utility of training a surgical technique on human cadavers. Scaglioni *et al*. dissected the pre-auricular skin of two fresh cadavers and then injected latex into the superficial temporal artery to identify perforators arising from this vessel.[29] A pedicled perforator flap based on these perforators was eventually designed. The experience gained with this training was applied successfully in 20 consecutive patients to reconstruct partial- and full-thickness defects of the anterior ear after basal or squamous cell carcinoma excision.

Type of human cadaver model

Data relevant to type of cadaver model are provided in Table 2. Most studies employed whole cadavers (*n* = 12)[28, 91-93, 95, 97, 168, 169, 176-179] or partial bodies (*i.e.* heads, brains, extremities, flaps and aortas) (*n* = 12)[29, 30, 59, 170-175, 180-182]. Three studies used combinations of whole and partial bodies.[94, 96, 183] Surgery was practised on the following body parts: head (*n* = 8)[94, 96, 170-174, 183], brain (*n* = 1)[175], aorta (*n* = 3)[180-182], extremities (*n* = 2)[94, 183] and flaps (*n* = 3)[29, 30, 59]. The number of reperfused body specimens used in individual studies ranged from one to more than 200.[97, 172] Thirteen studies each utilised fresh bodies[29, 59, 92, 94, 96, 97, 176, 178-183] and embalmed cadavers[28, 59, 91, 93-95, 170-175, 177] and three studies used both[59, 95, 170]. Several preservation methods were reported: formaldehyde (*n* = 9)[93, 95, 170-175, 177], Thiel embalming (*n* = 3)[28, 59, 91] and light (no details specified; *n* = 1)[170]. The authors of three publications did not report on preservation technique.[30, 168, 169]

Table 2. *Characteristics of reperfused human cadaver models. ALT, anterolateral thigh flaps; AW, abdominal wall flaps; IC, iliac crest flaps; n.r., not reported; OC, osteocutaneous flaps; STAP, superficial temporal artery perforator flap; WB, whole bodies.*

Study	Reperfused body or body part (*n*)	Status
Aboud *et al.*[170]	Heads (8)	Formaldehyde, partially fixed
Aboud *et al.*[183]	WB (4) heads (25), arms (4) legs (2)	Fresh
Aboud and Moursi.[96]	WB (11) and heads (13)	Fresh
Aboud *et al.*[95]	WB (14)	Fresh and formaldehyde fixed
Arbatli *et al.*[176]	WB (2)	Fresh
Bouma *et al.*[28]	WB (n.r.)	Thiel
Canaud *et al.*[180]	Descending thoracic aorta (15)	Fresh
Canaud *et al.*[181]	Descending thoracic aorta (8)	Fresh
Carey *et al.*[94]	WB, heads and extremities (38)	Fresh
Chapter 11 of this thesis	WB (8)	Thiel
Faure *et al.*[182]	Descending thoracic aorta (15)	Fresh
Garrett[97]	WB (> 200)	Fresh
Gragnaniello *et al.*[171]	Heads (6)	Formaldehyde
Güvencer *et al.*[172]	Heads (1)	Formaldehyde
Jongkind *et al.*[177]	WB (3)	Formaldehyde
Kawashima *et al.*[168]	WB (25)	n.r.
Kawashima *et al.*[169]	WB (22)	n.r.
Linsen *et al.*[178]	WB (6)	Fresh
Malikov *et al.*[30]	Thoracodorsal flaps (32)	Fresh

Study	Reperfused body or body part (*n*)	Status
Numan *et al.*[179]	WB (2)	Fresh
Olabe et al.[175]	Heads (4)	Formaldehyde
Olabe *et al.*[173]	Brains (4)	Formaldehyde
Pham *et al.*[92]	WB (8)	Fresh
Russin *et al.*[93]	WB (n.r.)	Formaldehyde
Scaglioni *et al.*[29]	STAP (4)	Fresh
van Doormaal *et al.*[174]	Heads (4)	Formaldehyde
Wolff *et al.*[59]	Radial forearm, ALT, OC, IC AW (48)	Fresh (15) and Thiel WB (6)

Vascular reperfusion technique

Most studies employed pump-driven reperfusion (**Appendix 5**).[28, 29, 59, 91-97, 170-172, 176-183] Alternative techniques included manual injection (*n* = 2)[29, 30] and drip regulators (*n* = 2)[173, 175]. Two publications did not specify how solutions were administered.[168, 169]

The arterial and venous systems were reperfused in 10 studies[59, 93-96, 168-170, 172, 183], but in most reports (*n* = 16)[29, 30, 91, 92, 97, 171, 173-182] only arteries were filled. In one case, the perfusate was simply pressurised in the left ventricle.[28] Arteries and/or veins were pressurised in 16 studies[28-30, 91-94, 96, 168-171, 173-175, 183], whereas arterial flow was generated in 11 studies[59, 95, 97, 172, 176-182]. One report described separate arterial and venous flows in a single isolated head.[172] After bilateral cannulation, the carotid artery and jugular vein were filled unilaterally with differently coloured perfusates, which eventually drained via the contralateral carotid artery and jugular vein, respectively. Other researchers used the same reperfusion technique, but the contralateral vessels that served as outflow were clamped or filled, creating pressurised vessels.[96, 170, 171, 173-175, 183] Several techniques were applied to reperfuse whole cadavers (Table 3). Sixteen researchers developed arterial pulsations, ranging from 35 to 120 pulsations per minute.[28, 59, 91, 95, 96, 170, 172, 174-178, 180-183]

Table 3. *Cannulation and reperfusion techniques. A, artery; Ao, aorta; CA, carotid artery; FA, femoral artery; FV, femoral vein; JV, jugular vein; n.a., not applicable; V, Vein; VA, vertebral artery; WB, whole body.*

Study	Specimen	Vessels	Reperfusion method	Inflow	Outflow	Vascular reperfusion
Malikov *et al.*[30]	Local	A	Pressurization	Axillary A	n.a.	n.a.
Scaglioni *et al.*[29]		A	Pressurization	External CA	n.a.	n.a.
Wolff *et al.*[59]		A, V	Flow	Feeding A to flap	Flap V(s)	Flow from A to V
Van Doormaal *et al.*[174], Gragnaniello *et al.*[171]	Head	A	Pressurization	Bilateral CA and VA	n.a.	n.a.
Aboud *et al*.[170], Aboud *et al.*[183], Aboud and Moursi[96] , Olabe *et al*.[175], Olabe *et al.*[173]		A, V	Pressurization	A: CA, VA; V: JV	Clamped	A and V separated
Guvencer *et al.*[172]		A, V	Flow	A: CA, VA; V: JV	Contralateral CA, VA and JV	A and V separated
Chapter 11 of this thesis, Pham *et al.*[92]	WB	A	Pressurization	FA, retrogradely	n.a.	n.a.
Russin *et al*[93], Carey *et al.*[94]		A, V	Pressurization	FA, retrogradely; FV, antegradely	n.a.	A and V separated
Aboud *and Moursi.*[96]		A, V	Pressurization; A: pulsatile, V: static	A: CA, V: JV	A: FA, V: FV	A and V separated
Garrett[97]		A	Flow	1.Descending thoracic Ao 2.Common or external iliac A	1.Superficial FA 2.Dorsalis pedis and posterior tibial A	Several flows from A to A
Aboud *et al.*[95]		A, V	Flow	CA, retrogradely	JV, retrogradely	Shunt between FA and FV

Water-based perfusion solutions were used in most studies (n = 20)[28, 59, 92-95, 97, 170-182]; latex (n = 1)[29], silicone (n = 4)[30, 168, 169, 171] and PL (n = 1)[91] were used less commonly. Nine studies involved the administration of a translucent perfusate[30, 91, 173, 175, 176, 179-182] and 10 reports described the injection of red solutions into arteries and veins[28, 29, 59, 92, 93, 97, 171, 174, 177, 178]. A few authors reported on the establishment of circulation with light-red solution in the arteries and dark-red solution in the veins[95, 170] or the filling of arteries and veins with red and blue solutions, respectively[94, 172]. Perfusate color and type were not described in two reports.[96, 183] Three publications described the use of latex or silicone, but did not specify whether these products were stained.[29, 168, 169]

Only five research groups established heated circulation.[91, 97, 172, 176, 179] In chapter 11 of this thesis we transferred and replaced aortic valves endovascularly in Thiel-embalmed cadavers using femoral access. Due to heat loss in the tubing system, PL was warmed to 50°C, allowing effective deployment of the valve. Other authors reported the use of saline solution at 37°C in a circulatory model for total endovascular repair of the aortic arch[176, 179] and one group used tap water at 22–24°C for neurosurgery training[172]. One publication did not report on the temperature of heated perfusate.[97]

Advantages and limitations of reperfused models

Many authors emphasised the immediate availability and high fidelity of human surgical training models compared with animal models (**Appendix 6**).[28, 92-97, 170, 172, 173, 176, 178, 182] Some researchers organised training in the treatment of pathological conditions, such as vascular diseases (*e.g.* dissection, aneurysm and catheter manipulation in calcified vessels)[91, 177, 182], artificial tumor resection[170, 171] and trauma[95, 96, 172]. In a few cases, various types of surgery were performed on the same body.[95-97, 170, 183] Some investigators preferred long-lasting, *i.e.* embalmed cadavers, which provided multiple training opportunities.[28, 59, 95, 172] A few authors emphasised the low cost of the training tools.[95, 96, 170, 175] Imaging modalities (*e.g.* radiography and ICG) that improved the realism of surgery and allowed the objective assessment of performance quality were appreciated.[29, 91, 94, 97, 173, 177, 178, 180-182]

Some authors described significant shortcomings of the models, such as single-session use of endovascular tools[97, 176], the lack of haemostasis[92, 170, 172-174, 182] and tissue deformation due to interstitial accumulation of perfusate[59, 94, 97, 175]. In addition, researchers noted that silicone hardens[30, 168, 169, 171] and the high viscosity of PL impedes contrast injection during endovascular treatments **(chapter 11)**. Few authors mentioned limited or absent venous circulation as a drawback.[59, 94] Some expressed concern about the cost of and time required for specimen preparation, which often necessitated an expert team.[93, 94, 181] A few publications described the difficulty of assessing the efficacy of surgical practise on human corpses (*e.g.* stents were placed in healthy aortas), which inevitably necessitated continued training on real patients.[94, 173, 180-182] With regard to body status, fresh cadavers are associated with health

hazards[94] and are usable only for brief periods[95]. Formaldehyde causes stiffness[95, 170, 174, 177, 182], alters tissue color and smells unpleasantly[95]. Thiel-embalmed cadavers are durable and useful for up to 1 year.[28]

Discussion

To better understand the current state of the art of reperfused human cadaver models, we performed an extensive literature review, demonstrating that reperfused human cadaver models may be excellent for training in advanced surgical techniques. Surgeons, residents and scrub nurses, as well as practitioners of other medical specialties, may benefit from learning (endo)vascular techniques under lifelike circumstances.[91, 95] Arterial reperfusion alone is sufficient for these procedures and lack of venous outflow is not a limitation. However, completely reperfused whole bodies are needed for training in trauma surgery or abdominal or thoracic resection.[95, 96] In addition, new applications for reperfused bodies, such as the surgical treatment of various simulated pathological conditions and assessment of graft perfusion completeness with ICG in flap raising procedures, have been reported, but are still in an experimental phase.

The studies included in the review were merely descriptive; a minority of publications focused on surgical training programmes and none of the authors organise workshops regularly. In addition, objective evaluation of individual skill progression and skills transfer to real life are rare. Bouma *et al*. required participants to achieve a minimum OSATS score to safely perform beating heart coronary anastomoses before proceeding to training on patients.[28] Efforts have been made to make it mandatory in the Dutch 6-year cardiothoracic surgery training programme at University Medical Center Groningen. Similar findings for human cadavers without reperfusion have been published recently. Gilbody *et al.* systematically reviewed the use of cadavers in workshops for post-graduate surgical trainees.[7] Of eight studies identified, two involved the assessment of skills transfer to patients after cadaver training and objective, reproducible assessments on patients were applied in only one study that evaluated the overall time of resuscitation. No study provided any evidence that skills learned by practising on cadavers improved OR performance. In three studies, subjective questionnaires revealed that trainees valued cadaver training. Despite trainees' and assessors' beliefs that cadaver workshops are useful adjuncts when teaching surgical skills, evidence for the transfer and cost-effectiveness of these workshops is limited.

Although filling the vessels of a complete body is complex, researchers reperfuse whole and partial bodies to the same extent and offer them for surgical practise. Most partial bodies comprised isolated heads and brains used for neurosurgical training. Occasionally, only partial-body reperfusion is needed for surgical training (*e.g.* flap raising, microvascular and endovascular procedures).

Generally, authors praised the immediate availability and high fidelity of cadaver models compared with the use of animals under general anesthesia. More than half of the included studies used fresh cadavers, which certainly have benefits, but these cadavers are only briefly available and their usage poses health risks. Not unexpectedly, formaldehyde-fixed cadavers were employed most frequently since this embalming method is used most commonly worldwide as shown in a survey.[49] This technique, however, causes tissue stiffness, discolouration and odours, hindering effective reperfusion, lifelike simulation and ease of use.[37] Strikingly, Thiel-embalmed cadavers were used for training in only three studies, showing that this embalming technique is not well known.[49] Thiel-embalmed specimens are soft and thus ideal for vascular reperfusion. Willaert *et al*. installed a long flow (*i.e.* 2 h) from artery to vein in Thiel-embalmed pig kidneys with viscous PP, demonstrating the feasibility of this model for surgical training **(chapter 8)**. Identical flows have been established successfully in Thiel-embalmed human kidneys, livers and extremities **(chapter 10)**. All of these specimens retained softness due to pressure-controlled circulation and limited accumulation of PP in the interstitial spaces. The color and consistency of Thiel-embalmed tissues are very realistic, the embalming mixture is largely odourless and the method achieves lengthy preservation. Negative aspects of this technique are the long preparation time and high cost of embalming process (*i.e.* in terms of chemical products and other materials). In our anatomical department, the cost of Thiel embalming is approximately € 760, about 25 times the price of the classic formaldehyde-based technique. With the inclusion of infrastructure, the cost may be as high as $ 1,200.[36] Bouma et al. reported that the total costs of one training day is approximately € 3,765.[28] This includes a fee for using the skills lab OR and 1 Thiel cadaver (€ 1,500), 2 scrub nurses and a supervising cardiothoracic surgeon (€ 765) and all materials and disposables (€ 1,500).

Most studies included in this review involved the use of a pump for perfusate administration. Certain pumps can produce arterial pulsations, increasing lifelike simulation. Notably, no realistic flow from artery to vein has yet been generated in complete human cadavers. Only Aboud *et al*. connected both systems and developed real flow using self-created arteriovenous shunts.[95] Generally, solely arterial reperfusion was sufficient to practise the intended surgical procedures, but venous filling was essential for the practising of complex procedures (*e.g.* trauma surgery and liver surgery).[95, 96] Researchers either pressurised the vascular system or installed flow in the vessels, causing significant bleeding in surgery-induced injury to vessels. In a circulatory system, however, higher flow rates can be obtained, causing more realistic haemorrhage.

Investigators reperfused vessels mainly with aqueous solutions, but the low viscosity of these solutions easily leads to extravasation and tissue deformation. Although flow can be generated with water-based solutions, viscous solutions such as PP, PL and PEG have long

intravascular retention times and extravasation depends on viscosity, molecular weight and reperfusion times.[82, 90, 185] For example, Grabherr *et al.* installed flow from artery to vein with PL in 45 fresh human cadavers for forensic diagnostic purposes.[90] Notably, only a few drops of PL were found in the gastric lumen in the vast majority of bodies. This limited leakage may be explained by the short reperfusion time (*i.e.* 6 min) and high viscosity of this product. Perfusate heating and the use of distinct colours or color gradation in arteries and veins are used to mimic real conditions.[28, 29, 59, 91-95, 97, 170-172, 174, 176-179]

The review process has limitations. Our search was limited to English-language publications because to our knowledge, no German national or regional electronic bibliographic database exists. Nevertheless, Thiel published originally in German, we would recommend a search of the German literature.

In conclusion, post-mortem reperfusion for advanced surgical training is feasible and offers great advantages in terms of fidelity. Despite the wide range of reperfusion techniques, realism of the human corpse is determined mainly by tissue status (*i.e.* fresh *vs*. embalmed). Reperfused human cadavers allow the development of realistic surgical training models and enable training mostly in cardiovascular procedures. New surgical applications will arise. Research should evaluate skills acquisition on reperfused human cadavers; transferability and cost-effectiveness prior to implement these advanced training models in structured stepwise proficiency-based training curricula.

Part C

Exploration and assessment of dynamic reperfusion in fresh pig tissues: two pilot studies

Chapter 6

Postmortem reperfusion of a pig: A first step to a new surgical training model?

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Abstract

Background The purpose of this experimental study was to establish a short-term postmortem circulation in a pig model using liquid paraffin. This study also investigated the quality of vascular perfusion in the peripheral tissues. This is the first step in the development of a new revascularized human surgical training model.

Methods This first experience was performed on the hindquarter of a pig. Initial cannulation of the external iliac artery and vein was followed by connection of the arterial inflow to a heart-lung machine and using the venous outflow to flush postmortem clots and blood. Subsequently, after connecting the venous outflow to the heart-lung machine, circulation was initiated.

Results Circulation was established during 27 minutes, during which the flow was constantly 130 mL/min. A steady increase in inlet pressure was observed during the experiment, which finally reached a minimum value of 124 mmHg. Perfusion was interrupted early due to an uncontrollable fluid leak. Afterwards, the distal part of the hind leg was incised showing an equal distribution of paraffin.

Conclusion A short-term revascularization was successfully re-established under excellent conditions. Although the results are promising, further experiments are necessary to eventually perform a wide range of surgical procedures on revascularized human cadavers.

Introduction

In recent decades, there has been a notable trend toward minimally invasive surgical techniques, which require a long learning curve. Therefore, a wide range of learning models have been developed as an alternative to training on patients. These models include laparoscopic VT, VR, anesthetized animals and human cadavers.[39, 186-188] However, surgical procedures performed on human corpses undoubtedly simulate best *in-vivo* conditions.[39]

In 2001, the first human cadaveric circulation model was reported.[97] Although this model has its merits, only flow in the arterial anatomy was established. Recently, a more realistic training model has been developed, using colored fluid under static and pulsating pressure for arteries and static pressure alone for veins.[96] A drawback of this model is the separation of both circulations.

Despite the usefulness of these circulation models, a realistic human training model must have a lifelike continuous flow in both the arterial and venous system. Grabherr has successfully re-established the total vascular circulation during a short period in deceased patients using a modified heart-lung machine.[90] However, this technique has only been performed for experimental and diagnostic purposes in forensic medicine.[87, 88, 90] Previously, the same author effected a long-term postmortem circulation in a cat and two dogs with odoriferous diesel oil.[87]

Therefore, the aim of the current study was to establish a lifelike short closed postmortem circulation in the right hindquarter of a pig using odorless paraffin oil.

Methods

Subject

This experiment was approved by the local ethics committee. We used the right hindquarter of a 6 month old pig. This specimen weighted 13.9 kg and was detached of the body in the slaughterhouse three hours before by roughly cutting through the skin; pelvic bones and muscles; and iliac vessels. Initially, the circumference of the ankle was measured. Afterwards, the external iliac artery and vein were prepared and cannulated, while the tubes were affixed with Silkam 0 sutures (B.Braun, Tuttlingen, Germany) (Fig. 1).

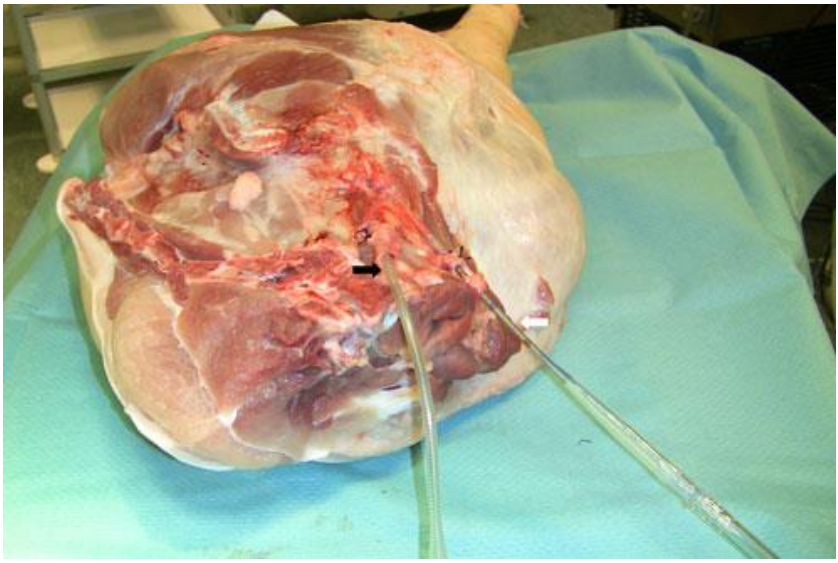

Figure 1. *The right hindquarter of a 6 month old pig. The tubes are inserted in the external iliac artery (white arrow) and vein (black arrow)*

Establishing a postmortem circulation

This was followed by priming the circuit with PP (Sigma-Aldrich, Bornem, Belgium). Subsequently, the arterial tube was connected to a heart-lung machine (Cobe, Sorin Group, Mirandola, Italy). Next, a board-certified clinical perfusion scientist started the perfusion via the arterial tube. The presence of perfusate containing postmortem clots and blood emerging from the cannulated iliac vein was considered to indicate a successful perfusion. Any leakage from small vessels was adequately clipped or sutured. The venous tube was then connected to the heart-lung machine when the perfusate was free of clots and blood.

Afterwards, postmortem circulation was established, during which leg circumference at the ankle (cm), perfusion time (min), perfusion inlet pressure (mmHg), flow rate (mL/min) and perfusion volume (mL) were measured every three minutes. This circulation was successful if the perfusion parameters (inlet pressure and flow rate) reached a steady-state.

Macroscopic inspection

Finally, after terminating the procedure, the skin and subcutis at the ankle were incised to evaluate the presence of PP.

Results

Establishing a postmortem circulation

Half a liter of PP adequately flushed most remaining blood and postmortem clots. However, PP diffusely leaked from the raw muscle surface and sawn sacral bone marrow. This leakage was uncontrollable but moderate and diminished after initiating circulation, which was effected during 27 minutes at a constant flow rate of 130 mL/min (Fig. 2). This circulation was interrupted early due to the persistent leak, causing a considerable reduction in circulating volume. The results of the perfusion parameters during this procedure are presented in figures 3 and 4.

The circumference of the ankle did not change. Moreover, the appearance of the external iliac vein remained physiological without signs of high wall tension (Fig. 2).

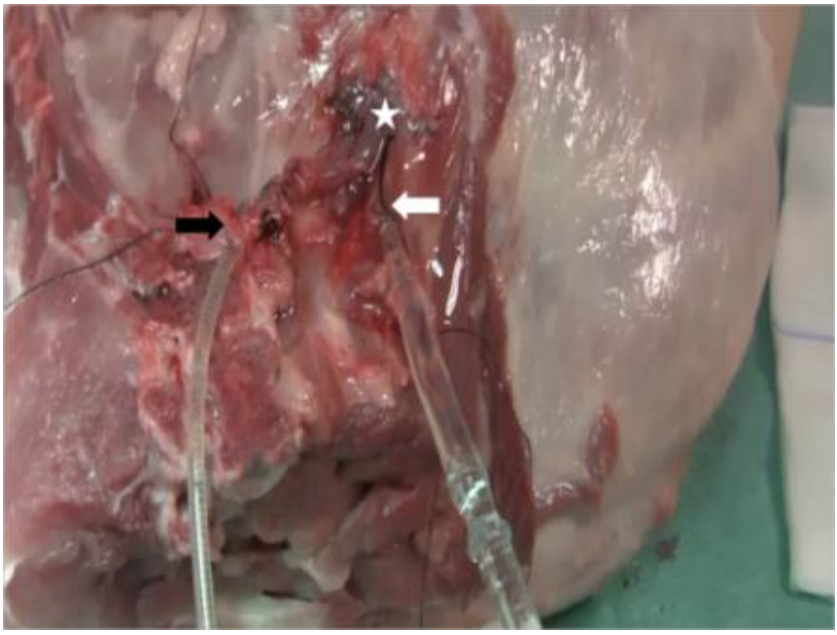

Figure 2. *The glossy raw surface of the muscles during the circulation due to diffuse leakage of PP. The tubes are inserted in the external iliac artery (white arrow) and vein (black arrow). The asterisk shows physiological swelling of the external iliac vein.*

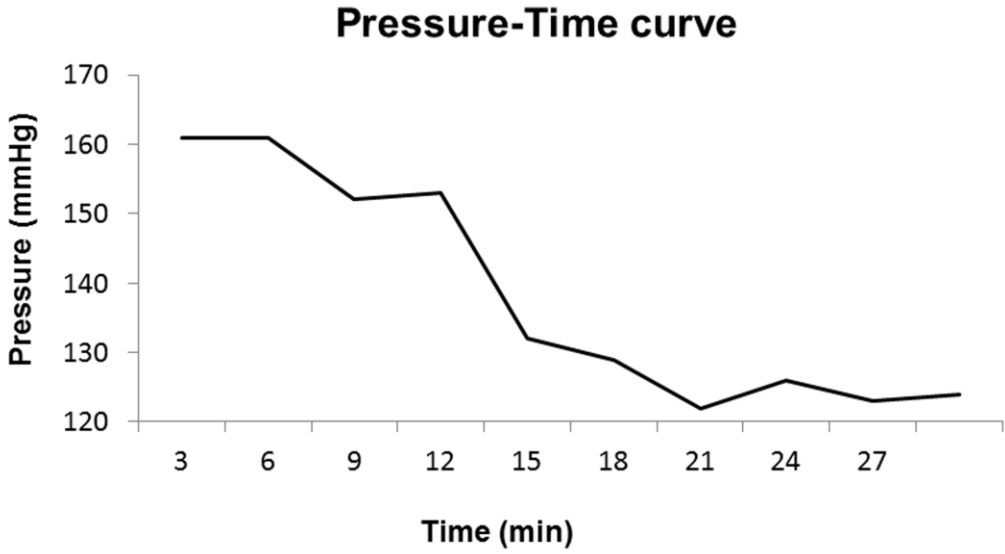

Figure 3. *A diminishment of the perfusion pressure from 161 mmHg at the beginning of the circulation to a steady-state of 124 mmHg at the end.*

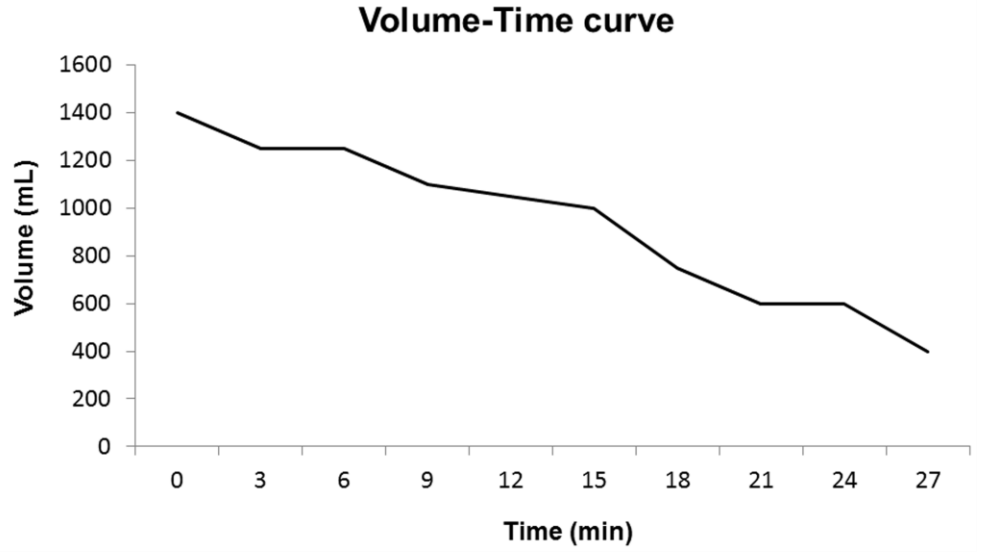

Figure 4. *Loss of 1,000 mL perfusion volume during the circulation.*

Macroscopic inspection

Finally, after terminating the perfusion procedure, the distal part of the hind leg was incised, showing equal distribution of PP in the tissues and some small amounts of remaining blood (Fig.5).

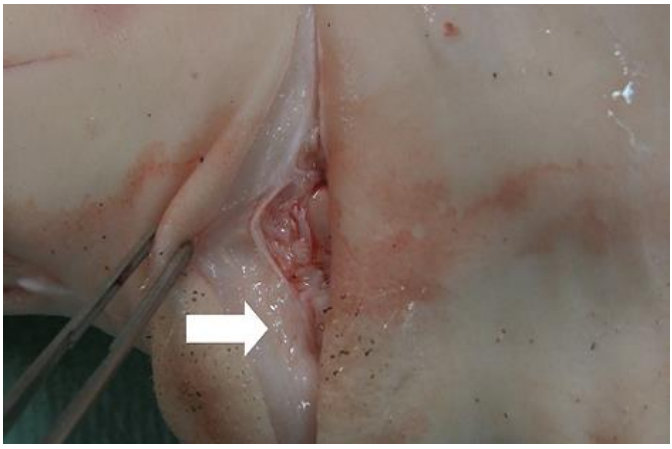

Figure 5. *PP and some blood at the distal part of the hind leg. PP has a glossy appearance (white arrow). Edema is absent.*

Discussion

The purpose of this experiment was the establishment of a continuous lifelike closed postmortem circulation in the right hindquarter of a pig. The hindquarter was chosen because its supplying iliac vessels have a considerable lumen making an adequate flushing and subsequent circulation possible.

A persistent perfusion during 27 minutes was successfully effected. To our knowledge, this is the first report describing short-term pump driven reperfusion of the total vascular system with PP. Similarly, diesel oil has been used to establish long-term postmortem circulation but its strong odor makes it unusable for surgical training models. Moreover, perfusion parameters are not reported in this experiment.[87]

Remarkably, this study approaches unique lifelike conditions as the perfusion pressure, after an initial increase, diminishes during the circulation to reach a steady-state of 124 mmHg. In accordance to the literature, this value will not damage the wall of the perfused vessels.[189] This finding is affirmed by the physiological appearance of the external iliac vein and the absence of edema at the ankle.

Not surprisingly, the constant flow rate of 130 mL/min during the circulation is lower than in human beings. Interestingly, the course of the perfusate through the tubes is clearly visible and fast, which is ideal for a future surgical training model.

The presence of PP at the ankle demonstrates its important properties. Nevertheless, some small vessels contain blood, which can be tackled by using less viscous perfusate. The viscosity of PP, which is 31 mPa.s (certificate of analysis, Fluka Analytical, Buchs, Switzerland) can be diminished by adding an alkane, resulting in perfusion of smaller vessels. Note that a perfusate with a viscosity lower than 18 mPa.s needs to be avoided because

perfusion of the capillary system results in edema.[185] Establishing a circulation using more viscous perfusate like PL, which we previously tested in a pig model, is not a valuable alternative for PP because it necessitated too high inlet pressure resulting in low flow rates (data not published).

Unfortunately, the circulation was interrupted early due to loss of perfusion volume. The rate of this loss was lower during circulation compared with the flushing period, due to pump-induced suction in the lumen of the venous tube. This loss can have several causes. Firstly, but probably least importantly, owing to recruitment of extra vessels during circulation. This phenomenon might play a role at the beginning, but may be limited because it mainly involves small blood vessels with a higher opening pressure. Secondly, rupture of the vessels can cause leak, which is unlikely because of the physiological appearance of the external iliac vein and the absence of edema at the ankle. Thirdly, leaks at the level of the cutting edges of the muscle surface and the hemisectioned sacrum are considered the main cause of the observed volume loss, which can be easily tackled by using a total body.

It should be borne in mind that PP is colorless, which is a disadvantage. Adding a fat-soluble red dye can color PP to mimic blood.[88]

A possible limitation of this experimental study is the use of only one specimen. However, postmortem revascularization of animals and full human bodies during a few minutes for diagnostic purposes has been reported in large series in the literature.[87, 90] Thus, the authors mean that by successfully establishing a longer reperfusion in a small part of one body proves the possibilities of this experimental model making the use of a series of hindquarters superfluous. Nevertheless, further research is necessary to investigate if PP circulates under the same conditions in an organ or the total body. Moreover, the microscopic circulation and possible adverse effects of prolonged reperfusion need to be evaluated.

In conclusion, PP enables a short-term reperfusion of the vascular system under ideal conditions. Both the heart-lung machine and PP can form a unique combination in the future development of revascularized human surgical training models, enabling to make vascular reconstructions and to handle vessels during minimally invasive procedures in the thorax and abdomen.

Chapter 7

Postmortem pump-driven reperfusion of the vascular system of porcine lungs: Toward a new model for surgical training

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Abstract

Background The objective of this experiment is to establish a continuous postmortem circulation in the vascular system of porcine lungs and to evaluate the pulmonary distribution of the perfusate. This research is performed in the bigger scope of a revascularization project of Thiel embalmed specimens. This technique enables teaching anatomy, practicing surgical procedures and doing research under lifelike circumstances.

Methods After cannulation of the pulmonary trunk and the left atrium, the vascular system was flushed with PP through a heart-lung machine. A continuous circulation was then established using red PP, during which perfusion parameters were measured. The distribution of contrast-containing PP in the pulmonary circulation was visualized on CT. Finally, the amount of leakage from the vascular system was calculated.

Results A reperfusion of the vascular system was initiated during 37 minutes. The flow rate ranged between 80-130 mL/min throughout the experiment with acceptable perfusion pressures (range: 37-78 mmHg). CT imaging and 3D reconstruction revealed a diffuse vascular distribution of PP and a decreasing vascularization ratio in cranial direction. A self-limiting leakage (i.e. 66.8 % of the circulating volume) toward the tracheobronchial tree due to vessel rupture was also measured.

Conclusion PP enables a circulation in an isolated porcine lung model with an acceptable pressure-flow relationship resulting in an excellent recruitment of the vascular system. Despite these promising results, rupture of vessel walls may have caused leak. Further exploration of the perfusion capacities of PP in other organs is necessary. Eventually, this could lead to the development of reperfused Thiel embalmed human bodies, which have several applications.

Introduction

Over the last twenty years, many surgical procedures have evolved toward more minimally invasive techniques, which require high-level skills to treat patients safely.[190] To improve these skills, a wide number of learning models have been developed.[39, 42, 44, 71, 186-188]

Surgical procedures performed on human cadavers undoubtedly simulate the best *in vivo* conditions.[39, 42, 44, 71] However, most of these cadavers lack a circulation, which excludes simulation of local bleeding. In 2001, Garrett established a pump-induced flow with a red crystalloid solution in several isolated arterial circuits of embalmed cadavers.[97] Accordingly, Arbatli pumped warm saline through an isolated part of the aorta of two fresh cadavers.[176] Güvencer made the model more complex and added a separated circuit in the venous system of formalin-fixed heads.[172] Recently, Russin pressurized entire cadavers via the femoral vessels to perform extracranial-to-intracranial bypass.[93] Aboud used a machine which provides a pulsating pressure in the arterial system and a steady pressure in the venous system. Note that in this model which has been tested in pressurized cadaveric heads as well as total human cadavers both vascular circuits are separated and no flow has been installed.[96, 170, 171] Recently, however, the same author elaborated this technique and established a semi lifelike circulation by performing arteriovenous shunts in fresh and formalin-fixed bodies.[95] Interestingly, Grabherr has installed a brief continuous circulation in the vascular system using viscous paraffin oil. This technique has been used for experimental and diagnostic purposes in forensic medicine.[88, 90]

To date, despite a wide set of revascularized training models, a long-lasting continuous flow in both vascular systems mimicking lifelike conditions is still absent. Thiel embalmed human bodies seem promising as a model for revascularization studies due to the authentic soft tissue preservation. Thus, in the context of our revascularization project, as part of an advanced surgical training program, this study is set up to reperfuse the porcine pulmonary system in controlled circumstances.

Therefore, the purpose of this experiment was to establish a continuous, long-lasting circulation in the vascular system of fresh pig lungs and to evaluate the pulmonary distribution of the perfusate.

Materials and methods

Animal model

The current experiment was approved by the local animal ethical committee (approval code 11/36). One pair of fresh pig lungs connected with the heart was obtained from the slaughterhouse. Lungs were chosen because these organs contain arteriovenous shunts.[191] The heart was removed, but the posterior wall of the left atrium remained connected with the pulmonary veins. Cannulas equivalent to the size of the pulmonary trunk as well as the left atrium were inserted and affixed. A flexible plastic tube was placed in the trachea to insufflate this pair of lungs. In case of an air leak, tears in the visceral pleura were adequately repaired.

Establishing a postmortem circulation

Then, this pair of lungs was placed in a plastic box. The inflow cannula was connected to a heart-lung machine (Cobe, Sorin Group, Mirandola, Italy), previously primed with PP (Sigma-Aldrich, Bornem, Belgium). Thereafter, a board-certified clinical perfusion scientist started extracorporeal reperfusion, while measuring the flow rate (mL/min), pressure (mmHg) and resistance (mmHg.min/L). During this reperfusion, any remaining clot or blood was drained through the venous cannula in a reservoir. At the same time, observed leaks were clipped or ligated. The reperfusion was terminated once venous drainage was free of blood.

Subsequently, this pair of lungs was mounted in a rectangular Plexiglas tank of 33.6 L containing four horizontally fixed wooden bars. Two bars were fixed at the bottom, while the others were superficially positioned. The base of both lungs and the trachea were affixed to the bars. The tank was then filled with 10,000 mL of PP.

To facilitate visualization, 150 mg Oil Red O (Sigma-Aldrich, Bornem, Belgium) was mixed with 1,700 mL of PP (*i.e.* 88 mg/L). Next, the venous cannula was connected to the heart-lung machine to establish a closed reperfusion with red PP, during which perfusion pressure (mmHg), resistance (mmHg.min/L), volume (mL) and flow rate (mL/min) were measured at fixed times. The mean arterial blood pressure of adult pigs *in vivo* is approximately 90 mmHg (unpublished data). Therefore, to minimize vascular damage, lower arterial blood pressures were intended. The volume of PP in the tank was measured after terminating the circulation. Eight samples were taken for spectrophotometric calculation of the concentration of Oil Red O (mg/mL) in the tank. These data were then used to calculate the total mass of Oil Red O (mg) lost during the reperfusion toward the tank.

Distribution of the perfusate

After re-establishing perfusion, a bolus of 90 mL Angiofil®, a liposoluble contrast agent, was injected in the arterial cannula. When Angiofil® reached the venous cannula, the circulation was again interrupted and the specimen was brought to the CT (Somatom Definition Flash, Siemens Healthcare Sector, Forchheim, Germany) to visualize the pulmonary course of PP (slice thickness: 1 mm, kV: 100, Mas: 134, FOV 512x512 (266mm x 266mm), convolution kernel: B70f). This was followed by a 3D reconstruction of this pair of lungs with Mimics 15.0, based on the 2D CT data stack. An automated mask was created using a threshold between 368 and 3071 Hounsfield units, enabling a thorough segmentation of the vascular contrast medium. The visceral pleura that was included in the applied segmentation was manually removed to allow clear visualization of the vascular tree. A high quality 3D reconstruction was calculated from the processed final segmentation.

Next, ten axial planes with 30 mm inter-distance were depicted in the reconstruction in a cranial-to-caudal sequence. Measurements were performed at each axial level.

Firstly, the mean smallest vascular diameter was determined by measuring the main cross-sectional diameter of the blood vessels at ten to twelve uniformly distributed locations at the peripheral border of each axial plane. Subsequently, the amount of vascular tissue was determined by calculating the relative vascularization area in each of the ten axial planes. Using a Boolean function the vascular tissue was subtracted from the total surface area at each axial level. As a result, the total vascular surface area could be calculated and expressed as a percentage of the total surface area of that level. This number is an indication of the relative amount of vascular tissue that can be visualized using the current CT and segmentation techniques. Next, the total volume of the contrast medium filling up the vascular tree was calculated and expressed in milliliter. Afterwards, the specimen was cut into slices to observe the macroscopic distribution of PP. Finally, eight samples were taken from the base, apex, lateral and medial surface of both lungs.

Calculation of the leakage into the tank

Firstly, 10 dilutions ranging from 1 g to 1 mg Oil Red O/L PP were made. Secondly, with a spectrophotometer, (Shimadzu UV Visible 1800, ‘s-Hertogenbosch, The Netherlands) the absorbance of each sample was measured at a wavelength of 518 nm, which is the absorption maximum of Oil Red O. Thirdly, the absorption coefficient of each sample was calculated with the Lambert-Beer law ($\varepsilon = E/C \times L$, where ε, E, C and L represent the absorption coefficient (L/mol x cm), absorbance (no unit), molar concentration (mol/L) and path length in cuvette (cm), respectively) and was then used to determine the mean absorption coefficient of Oil Red O. Next, the absorbance of eight samples taken from the

tank was measured. The average leakage (mg) of Oil Red O toward the tank was eventually calculated using the Lambert-Beer law and the mean absorption coefficient.

Results

Establishing a postmortem circulation

During eight minutes, 800 mL of PP was perfused into the arterial cannula, of which 350 mL flushed a small amount of remaining blood and postmortem clots via the venous cannula. Due to vascular leaks, 80 mL of PP was lost into the plastic box. These leaks were adequately sutured. Thus, after flushing, 370 mL of PP remained in both lungs and cannulas (Fig. 1a). After suspending the specimen in the tank, a continuous circulation with 1,700 mL red PP was initiated for 37 minutes, during which 1,175 mL was lost into both the tank and the tracheobronchial tree. In detail, 40 mL red PP (*i.e.* 2.3 %) entered the tank, resulting in a volume of 10,040 mL PP at the end of the reperfusion procedure. As a consequence, 1,135 cc mL or 66.8 % of the total circulating volume must be lost toward the tracheobronchial system (Fig. 1b). Flow rate, inlet pressures and resistance during the reperfusion phase are illustrated in figure 2.

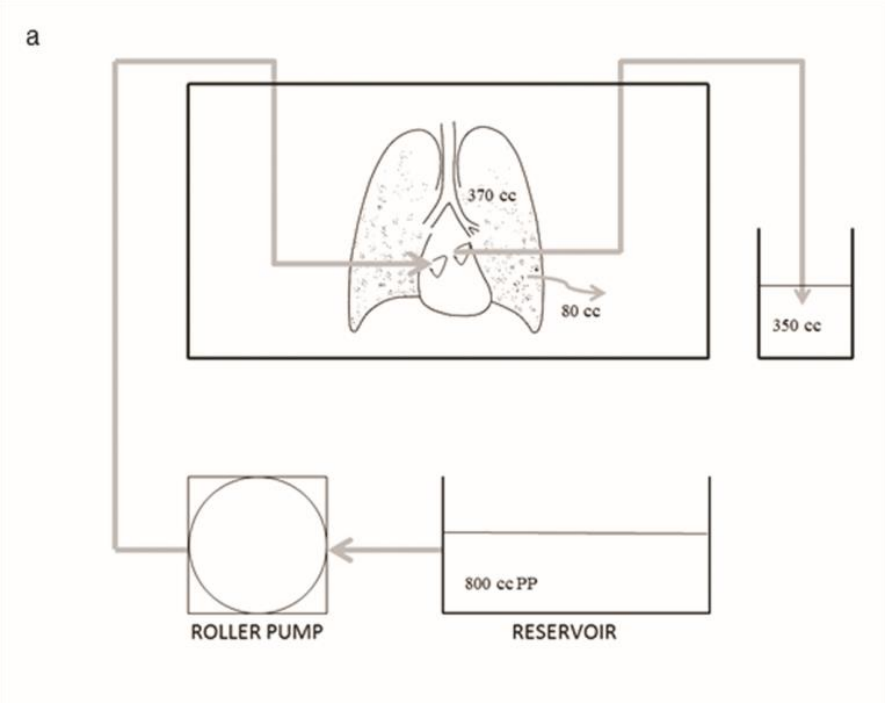

Figure 1a. *Flushing model. Using the arterial cannula, 800 mL of PP was inserted in a pair of lungs, of which 350 mL flushed any remaining blood and postmortem clots via the venous cannula. Eighty mL was lost via vascular leaks, whereas 370 mL remained in both lungs and cannulas.*

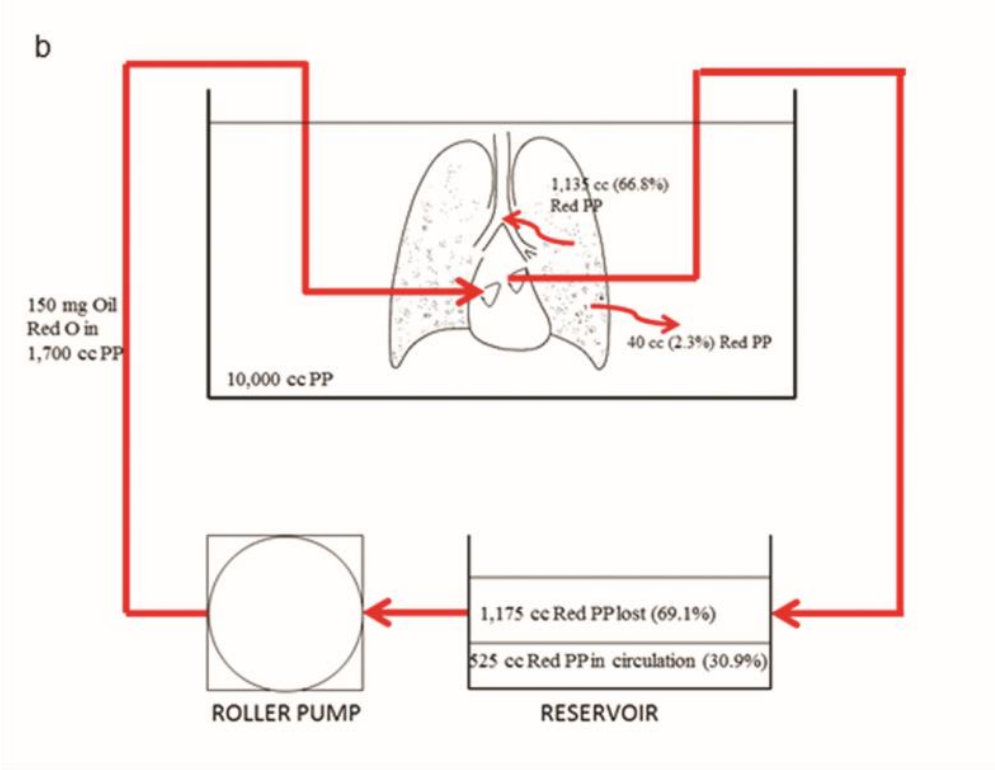

Figure 1b. *Reperfusion model. After connecting the venous cannula to the pump a continuous reperfusion was installed with 1,700 mL Red PP. A small amount (i.e. 40 mL) was lost toward a tank filled with 10,000 mL PP, while the majority leaked into the tracheobronchial tree (i.e.1,135 mL).*

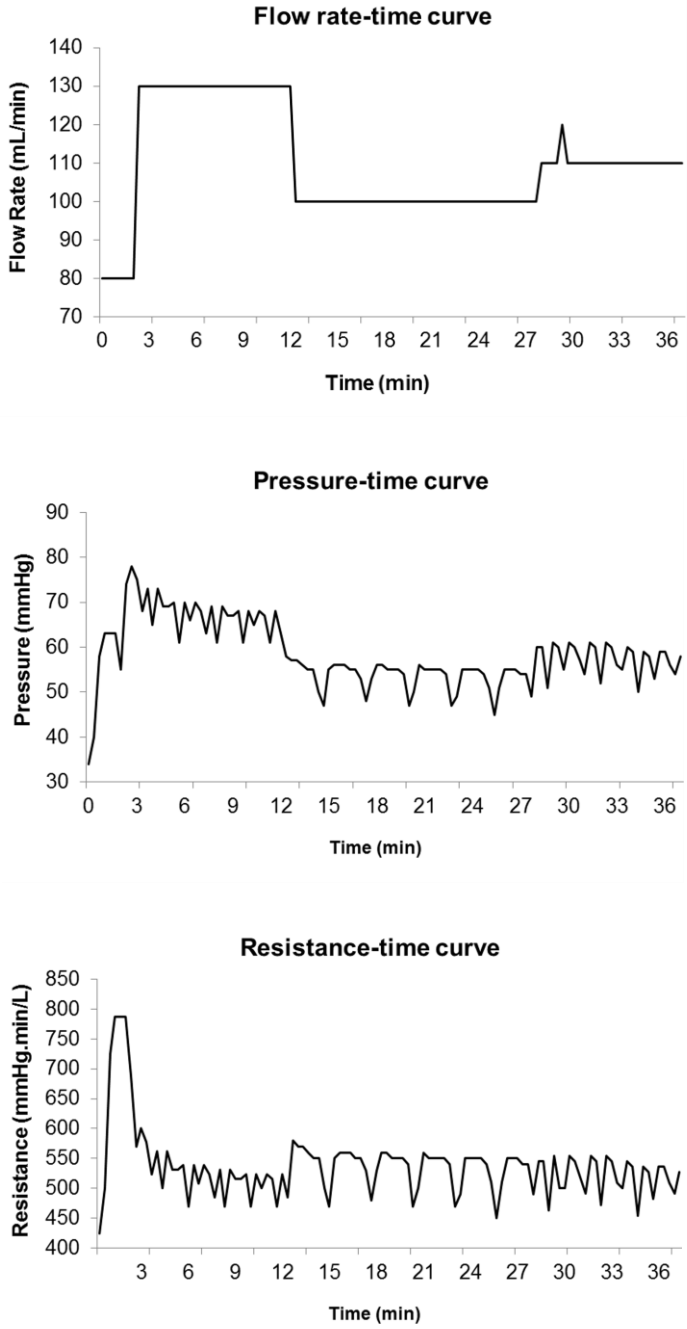

Figure 2. *Course of the perfusion parameters during circulation. The flow rate ranged from 80-130 mL/min, causing low inlet pressures (range: 37-78 mmHg) and resistances (range: 425-788).*

Distribution of the perfusate

Figure 3 depicts the vascular distribution of the porcine lungs in 3D. At first glance, it immediately strikes that the cranial bifurcations of the pulmonary trunk (posterior, 22 French tube) and the pulmonary veins (anterior, 32 French tube) are less dens in comparison to the caudal bifurcations. This initial observation is in correspondence with the measurements of the vascular diameter and the relative vascularization area.

As shown in Figure 4 (upper graph), morphometric measurements found a mean vascular diameter of 0.81 mm ± 0.13 (2 SD) with a decreasing trend in cranial direction (from slice 1 to 10; 0.88 mm - 0.75 mm).

Fig 5 displays the subtraction of the vascular tissue from the total surface area at each level. The mean relative vascularization area is 7.95 % ± 3.91 (2 SD). The trend line in the lower graph of Figure 4 also describes a decreasing vascularization ratio in cranial direction primarily due to the supracardiac drop of the vascularization area (from slice 1 to 10; 10.26 % - 5.63 %). The total amount of contrast fluid present in the porcine vascular tree, as presented in the 3D illustration, was 133.09 mL.

Inspection of the sliced lungs confirmed the widespread distribution of red PP (fig 6). Histology showed red blood cells in the capillaries without dilatation or congestion of the alveolar septae (fig 7).

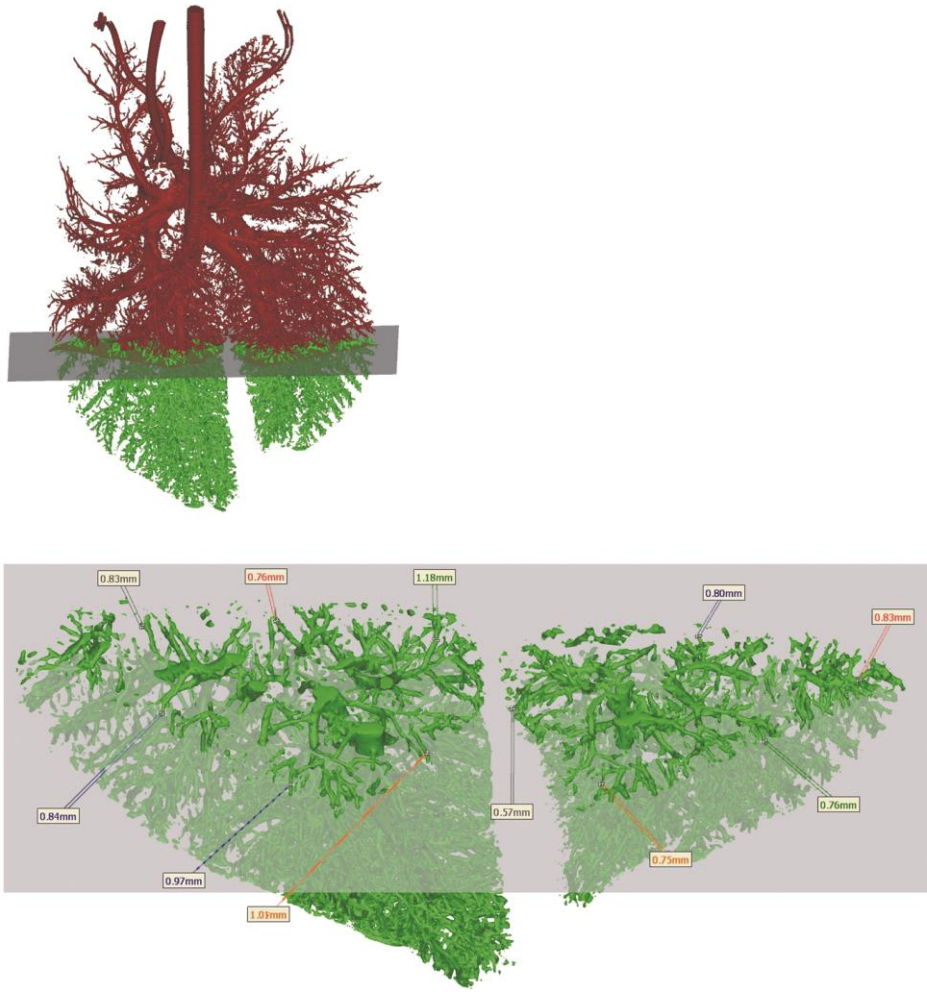

Figure 3. *Overview of slice 4 in the porcine vascular lung model. The area cranially to the cutting plane (slice 4) is removed to allow proper measurement of the vascular diameter of the most pheriperal vessels in the slice, as illustrated in the lower part of the figure.*

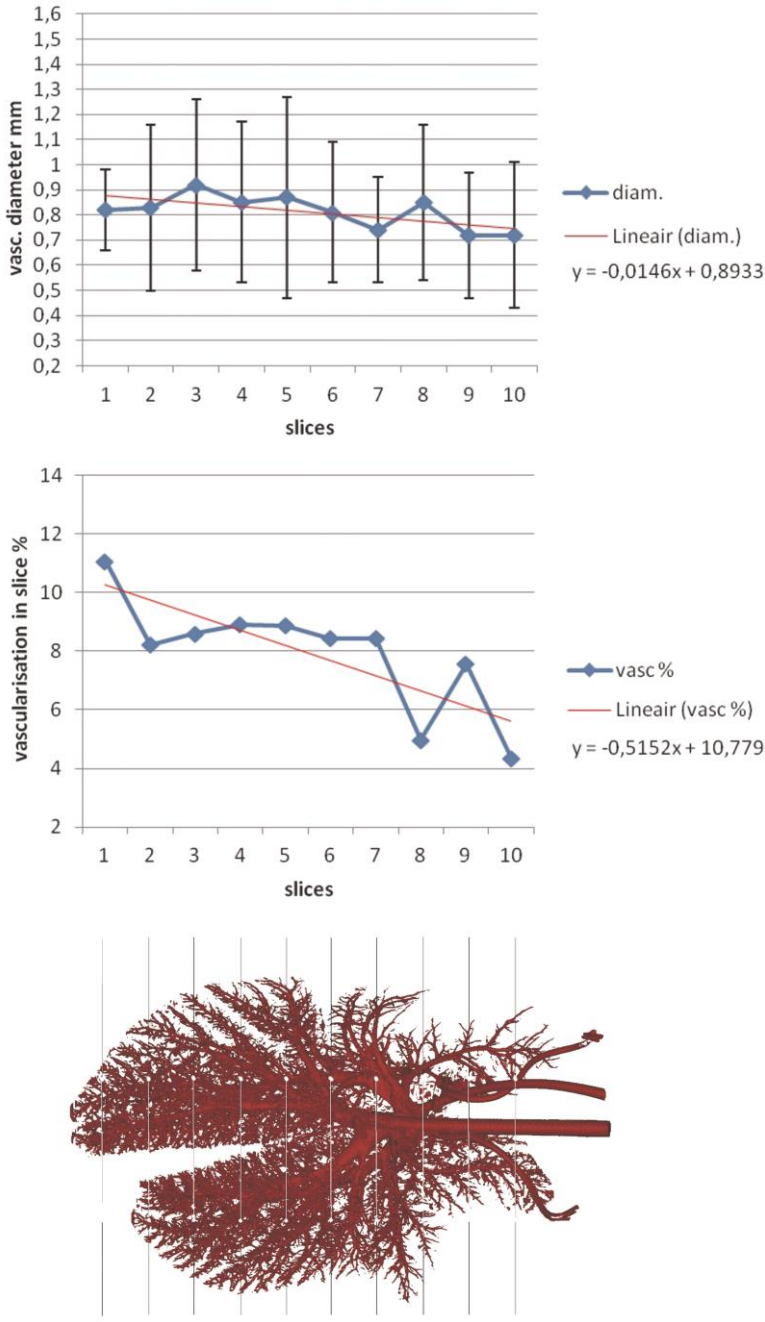

Figure 4. *Overview of the measurements of the vascular diameter and vascularization ratio. Upper graph shows the mean vascular diameter (mm) for each of the ten slices through the 3D reconstruction. The lower graph demonstrates the vascularization ratio (%) for each slice measured. Illustration at the bottom is a ventral view of the pulmonary vascular tree orientated so that the longitudinal slices correspond with the slice numbers represented on the x-axes of the upper and lower graph.*

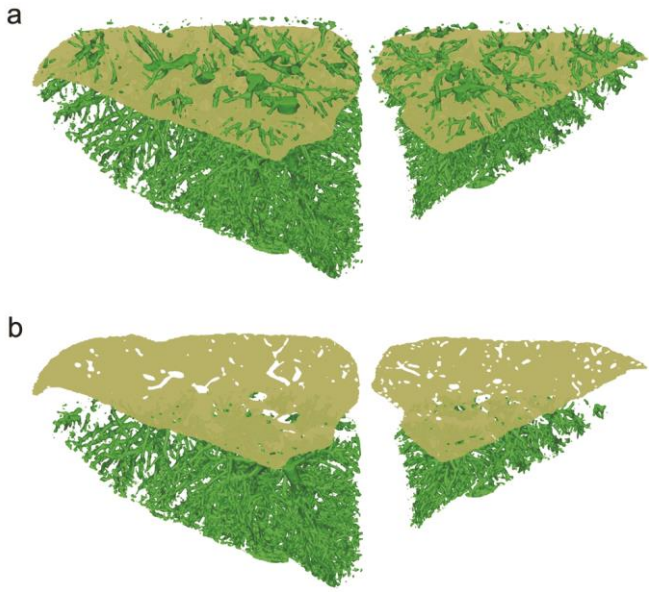

Figure 5. *Subtraction of the vascular tissue from the total surface area at each level. Total area of lung surface in slice 4 is delineated into a semi-transparent mask. Then, the corresponding vascular tissue extending cranially from the mask, is subtracted from this surface area with a Boolean function (**a**). In **b** the resulting surface area (semi-transparent) is illustrated; the white cavities show the contours of the subtracted vessels. Based on the surfaces as depicted in a and b, the total percentage of vascular tissue surface area in the particular slice can easily be determined.*

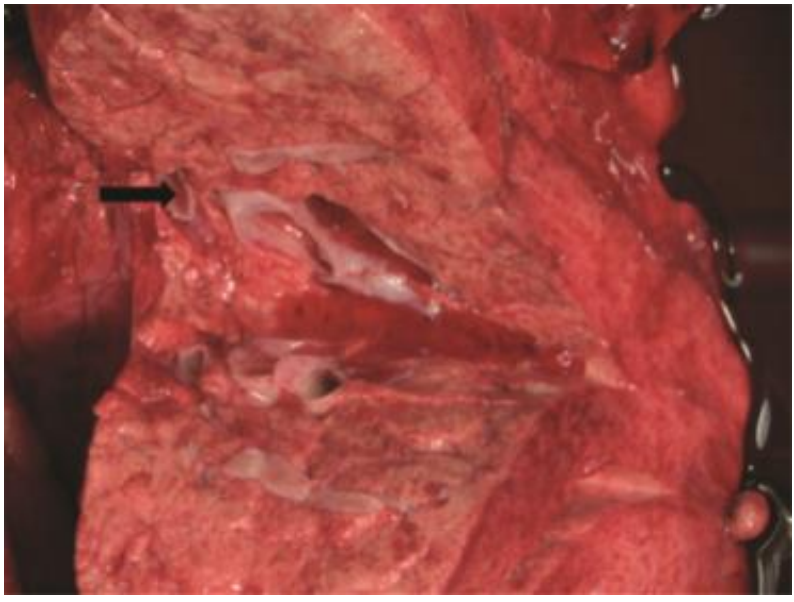

Figure 6. *Section through the right lung. The perfusate in the big vessels (arrow) and its diffuse spread across the parenchyma.*

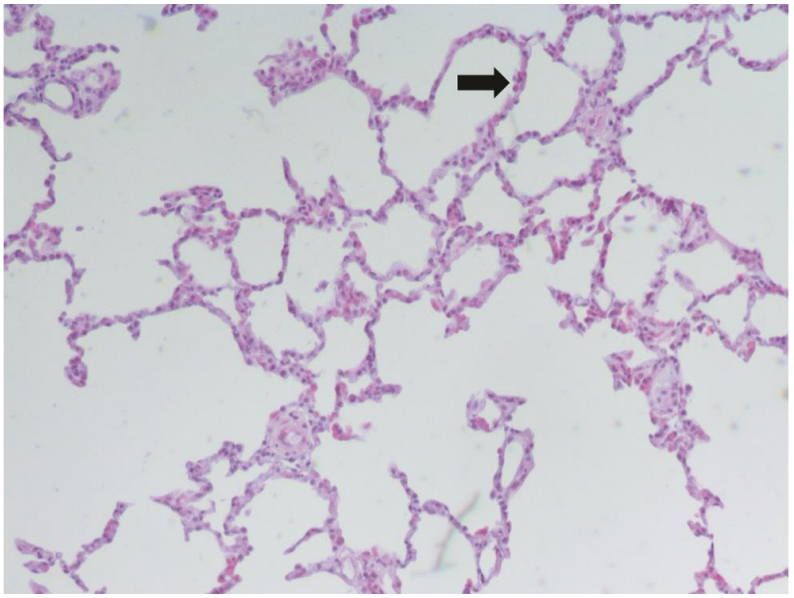

Figure 7. *H&E-stained histological specimen of both lungs. Red blood cells in the capillaries (black arrow), suggesting that the mixture does not enter the capillaries due to its viscosity of 31 mPa.s. There is no edema at capillary level because the alveolar septae are not dilated or congested with red blood cells.*

Calculation of the leakage into the tank

Table 1 demonstrates 10 dilutions of Oil Red O in PP; the measured absorbance and the calculated absorbance coefficient for each sample based on the Lambert-Beer law; and the mean absorbance coefficient of Oil Red O using samples 3 to 10.

Table 2 displays the absorbance and calculated concentration of Oil Red O for each sample taken from the tank; and the calculated mean concentration of Oil Red O in the tank being 0.371 mg/L. As a result, the amount of Oil Red O at the end of the circulation in the tank was 3.72 mg (*i.e.* 0.371 mg/L multiplied with 10,040 mL).

Table 1. *Concentrations of Oil Red O (mg/L) in the samples; the molar concentrations yielded by dividing the concentrations by the molar mass of Oil Red O (408.49 g/mol); the measured absorbance for each sample; the calculated absorption coefficient for each sample using the Lambert-Beer law; and the mean absorption coefficient based on the results of samples 3 to 10. The first two samples were not used because of spectrophotometric deviations of the measuring instrument. Mol = amount of a substance that contains as many elementary entities as there are atoms in 12 grams of carbon-12 (^{12}C); absorbance = a logarithmic measure of the amount of light absorbed (at particular wavelength) as the light passes through a sample or substance; absorbance coefficient = measurement of how strongly a chemical species absorbs light at a given wavelength.*

Cuvette	Concentration (mg/L)	Molar Concentration (mol/L)	Absorbance	Absorption Coefficient (L/mol x cm)
Blank Sample	0	0	0	
1	1000	2.448 x 10-3	3.41	1,392.974
2	100	0.2448 x 10-3	3.402	13,897.059
3	50	0.1224 x 10-3	2.064	16,862.745
4	25	61.2 x 10-6	1.044	17,058.824
5	12.5	30.6 x 10-6	0.553	18,071.895
6	10	24.48 x 10-6	0.43	17,565.359
7	5	12.24 x 10-6	0.209	17,075.163
8	2.5	6.12 x 10-6	0.111	18,137.254
9	1.25	3.06 x 10-6	0.054	17,647.059
10	1	2.448 x 10-6	0.04	16,339.869
Mean Absorption Coefficient				17,345

Table 2. *The measured absorbance for each sample taken from the reservoir; the mean absorption coefficient of Oil Red O; the calculated molar concentration for each sample using the Lambert-Beer law; the concentration of Oil Red O in each sample yielded by multiplying the molar concentration with the molar mass (408.49 g/mol) of Oil Red O; and the mean concentration of Oil Red O in the tank.*

Cuvette	Absorbance	Mean Absorption Coefficient (L/mol x cm)	Molar Concentration (mol/L)	Concentration (mg/L)
Blank sample	0	0	0	0
1	0.017	17,345	0.980 x 10-6	0.400
2	0.015	17,345	0.865 x 10-6	0.353
3	0.014	17,345	0.807 x 10-6	0.330
4	0.017	17,345	0.980 x 10-6	0.400
5	0.016	17,345	0.922 x 10-6	0.377
6	0.016	17,345	0.922 x 10-6	0.377
7	0.017	17,345	0.980 x 10-6	0.400
8	0.014	17,345	0.807 x 10-6	0.330
Mean Concentration			0.908 x 10-6	0.371

Discussion

The main purpose of this experiment was to mimic *in vivo* conditions by establishing a pump-driven circulation in the vascular system of one pair of fresh porcine lungs.

By combining both the pump and PP, we easily flushed residues of blood and clots. Strikingly, after connecting the venous cannula to the heart-lung machine, realistic reperfusion conditions in the vascular system were installed. These lifelike conditions are characterized by the significant flow rate of the perfusion mixture, its clearly visible passage through the cannulas and the relatively low inlet pressures ranging from 50-60 mmHg at the end of the procedure. Our team previously demonstrated the exceptional reperfusion properties of PP in the hindquarter of a deceased pig.[192] Similarly, Grabherr has successfully used diesel oil for long-term circulation to visualize the vascular system in animal models. However, no reperfusion parameters were reported.[87]

Besides acceptable reperfusion conditions, it should be noted that PP has other important properties to approach reality. This oily perfusate can be colored with Oil Red O, a fat-soluble red dye, to mimic blood.[87, 88] Moreover, CT images and macroscopic inspection of the sliced specimen demonstrate its complete distribution in the pulmonary parenchyma. Importantly, due to a viscosity of 31 mPa.s at room temperature (certificate of analysis, Fluka Analytical, Buchs, Switzerland), it uses arteriovenous shunts to bypass the capillary system. Consequently, capillary damage cannot be the cause of the leakage toward the tracheobronchial tree, because the alveolar septae are not dilated or congested with red blood cells. Grabherr has demonstrated that oil blocks the capillaries of chicken embryos, which are particularly vulnerable to postmortem permeability.[87] The intrinsic viscosity of vital blood is 4-5 mPa.s.[89] A minimal viscosity of at least threefold that of blood is recommended to prevent filling of the capillaries and subsequent tissue edema.[193] Therefore, a viscosity of 31 mPa.s seems sufficient to establish an ongoing circulation.

It should be borne in mind that the notable leakage toward the tracheobronchial tree suggests rupture of fragile small vessels. Probably, slightly too high inlet pressures are responsible for this phenomenon because the pulmonary vessel wall of the pig is usually exposed to lower pressures (*i.e.* ± 31 mmHg).[194] Thus, lowering the inlet pressure or adjusting the intrinsic viscosity of the perfusate could avoid or minimalize this leak. Using more viscous perfusate like we tried in a pig is not recommended because low flow rates needed too high inlet pressures, resulting in unavoidable rupture of vessels (unpublished observation).

An important disadvantage of red PP is its complete disappearance due to hydrophobic clearing agent used during the tissue processing. As a consequence, it is impossible to show PP in

biopsies and frozen sections. Perhaps, lipophilic fluorophores could be useful to visualize its microscopic level of reperfusion.

This experiment intended to measure the amount of loss from the vascular system. Spectrophotometric analysis showed that the smallest amount (*i.e.* 3.72 mg) of Oil Red O escaped toward the tank due to a small iatrogenic pleural shear. This finding emphasizes that PP probably does not have the capacity to break through the visceral pleura but could, due to its intrinsic viscosity and too high inlet pressures, rupture the vessels causing a major leakage as demonstrated on CT. Morphometric measurements show that vessels slightly smaller than 1 mm in diameter are filled with contrast agent. This is in accordance with the CT providing resolution, which is in the order of 1-2 mm. Calculation reveals that 133.09 mL contrast containing agent was present in the vascular system of this pair of lungs. Due to resolution limitations of the CT, this amount is probably slightly higher but, as the capillary system was not reperfused, certainly lower than the normal blood volume of a pair of pig lungs.
The 3D reconstruction also reveals that the contrast containing agent does not enter the airways, which demonstrates their complete filling during the pump-driven reperfusion. This finding suggests again that rupture of the vessels instead of edema must have caused a substantial leak.

The use of only one specimen could be considered as a limitation. However, repeating this procedure would not add any useful information because successful short-time reperfusion of animals, human limbs and total bodies has been described.[87, 88, 90] Moreover, this experiment and previous research demonstrate that even long-time reperfusion seems feasible.[192] Future studies are necessary to evaluate if reperfusion with PP is possible in a complete fresh body and Thiel embalmed organs.

This model could be useful to learn endovascular procedures as well as open vascular surgery. Moreover, the revascularization of thoracic and abdominal organs may allow to handle vessels and to control bleedings during minimally invasive surgery.

Conclusion

PP enables a continuous circulation in the vascular system of a pair of fresh pig lungs. Very realistic reperfusion conditions were established. Nevertheless, rupture of small vessel walls occurred. The present study provides a useful basis for the development of reperfused Thiel embalmed human bodies, which can have a wide range of applications.

PART D

The spread of Thiel embalming solution in pig kidneys and subsequent long dynamic reperfusion of these embalmed organs

Chapter 8

Understanding Thiel embalming in pig kidneys to develop a new circulation model

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Abstract

Background The quality of tissue preservation in Thiel embalmed bodies varies. Research on the administered embalming volume and its vascular distribution may elucidate one of the mechanisms of tissue preservation and allow for new applications of Thiel embalming.

Methods Vascular embalming with (group 1, *n* = 15) or without (group 2, *n* = 20) contrast agent was initiated in pig kidneys. The distribution of Thiel embalming solution in group 1 was visualized using CT. The kidneys in both groups were then immersed in concentrated salt solutions to reduce their weight and volume. Afterwards, to mimic a lifelike circulation in the vessels, group 2 underwent pump-driven reperfusion for 120 minutes with either PP or diluted PEG. The circulation was imaged with CT.

Results All of the kidneys were adequately preserved. The embalming solution spread diffusely in the kidney, but fluid accumulation was present. Subsequent immersion in concentrated salt solutions reduced weight ($P < 0.01$) and volume ($P < 0.01$). Reperfusion for 120 minutes was established in group 2. PP filled both major vessels and renal tissue, whereas diluted PEG spread widely in the kidney. There were no increases in weight ($P = 0.26$) and volume ($P = 0.79$); and pressure further decreased ($P = 0.032$) after more than 60 minutes of reperfusion with PP, whereas there were increases in weight ($P = 0.005$), volume ($P = 0.032$) and pressure ($P < 0.0001$) after reperfusion with diluted PEG.

Conclusion Arterial embalming of kidneys results in successful preservation due to complete parenchymatous spread. More research is needed to determine whether other factors affect embalming quality. Dehydration is an effective method to regain the organs' initial status. Prolonged vascular reperfusion with PP can be established in this model without increases in weight, volume and pressure.

Introduction

In 1992, Thiel reported a new soft embalming technique, which presently consists of vascular perfusion followed by immersion in a bath for at least two months.[32, 38] This technique is exceptional because the color, consistency and transparency of the tissues are very well preserved. Moreover, preservation is long-lasting and no harmful substances are released into the environment.[32] This soft-fix embalming technique provides a more realistic tool for surgical training when compared with formalin-embalmed cadavers.[44, 55] In addition, the bodies are more realistic than fresh-frozen material, which suffers from postmortem rigidity and putrefaction.[37, 71] As a result, Thiel cadavers are increasingly used for dissection courses, research purposes and training in several disciplines.[31, 37, 39, 40, 42-44, 47, 70, 71, 77]

Today, several issues concerning the Thiel embalming procedure are not standardized or remain unknown and unresolved. Consequently, there is not a standard technique to insure high quality tissue preservation. Thiel recommends a vascular embalming volume of 15.8 L for one complete cadaver.[32] This volume provides a sufficient distribution in the body, but is probably too abundant because we often observe the escape of embalming fluid via the ears and nose. Presumably, large amounts of fluid in the capillaries extravasate and accumulate in the extravascular tissues before eventually leaving the body. Thiel embalmed cadavers usually look slightly bloated.[37] Later, the fluid gradually drains out and the body returns to its pre-embalming appearance. Remarkably, this swelling, which suggests diffuse capillary spread of the embalming product, is often not associated with uniform, high quality preservation of the body.

Several issues must be resolved to perform solid embalming with Thiel embalming fluid. The vascular perfusion properties of Thiel embalming fluid have not yet been explored, which may explain why high quality tissue preservation is not always observed. Therefore, in this study, a kidney model was used to assess if incomplete vascular distribution of a fixed embalming volume is the causative factor for the observed variability in tissue preservation. Because embalming causes tissue swelling and deformation, an osmotic dehydration method was tested in Thiel embalmed kidneys to re-establish their original status in a fast and controlled way. Moreover, as part of a project to develop an ideal surgical training model of reperfusion in the vessels of Thiel embalmed human cadavers, we explored if a continuous pump-driven flow mimicking the circulation of blood can be established in the vessels of the dehydrated Thiel embalmed kidneys.

In this study, we demonstrate that variability in tissue embalming quality cannot be explained by insufficient vascular spread of a fixed embalming volume. In addition, embalming-induced swelling and deformation can be successfully halted by salt water immersion, which creates ideal circumstances for prolonged reperfusion of the renal vessels with PP.

Materials and Methods

This study was approved by the Committee on the Ethics of Animal Experiments of the University of Ghent, Belgium (approval code: 11/36).

Experiment 1

Fifteen fresh pig kidneys (group 1) were obtained from the slaughterhouse. Kidneys were chosen because they usually have only one main feeding artery and one draining vein, allowing to easily install a vascular circulation. Initially, the renal artery and vein were identified and the ureter was ligated. The specimens were then weighed and their volumes were measured by immersion in a vessel full of water (Archimedes' principle). Next, a Quik-Cath II 14-gauge catheter (Baxter, Mayo, Ireland) was placed in the renal artery and connected to a tube, which was placed in a roller pump (Watson-Marlow 520 U, Zwijnaarde, Belgium) that initiated Thiel embalming. The weight of the injected embalming solution was 22.7 % of the weight of the dissected kidney. This amount is in accordance with the administration of 18.170 kg of embalming solution (or 15.8 L) to a human cadaver of 80 kg as proposed by Thiel.[32] In addition, the volume of the administered embalming product was measured. Next, a contrast agent (Omnipaque 300®, GE Healthcare, Diegem, Belgium) was mixed with the embalming solution. The volume of contrast agent added was 10 % of the administered embalming volume.

After initiating the embalming procedure, the renal artery was punctured with a BD Insyte-W 22-gauge catheter (BD Vialon, Madrid, Spain) and connected to an ultraminiature fibre optic pressure transducer (Samba 201 CAP, Harvard Apparatus, Les Ulis, France). The maximum arterial pressure allowed was 90 mmHg, in agreement with the *in vivo* mean arterial blood pressure of pigs (Fig. 1). The type of venous drainage (transparent, serosanguinous or sanguinous) was assessed at the end of the embalming procedure. Subsequently, the weight, volume and swelling of the kidneys were noted. The vascular spread of the embalming product was imaged by CT (Somatom Definition Flash, Siemens Healthcare Sector, Forchheim, Germany).

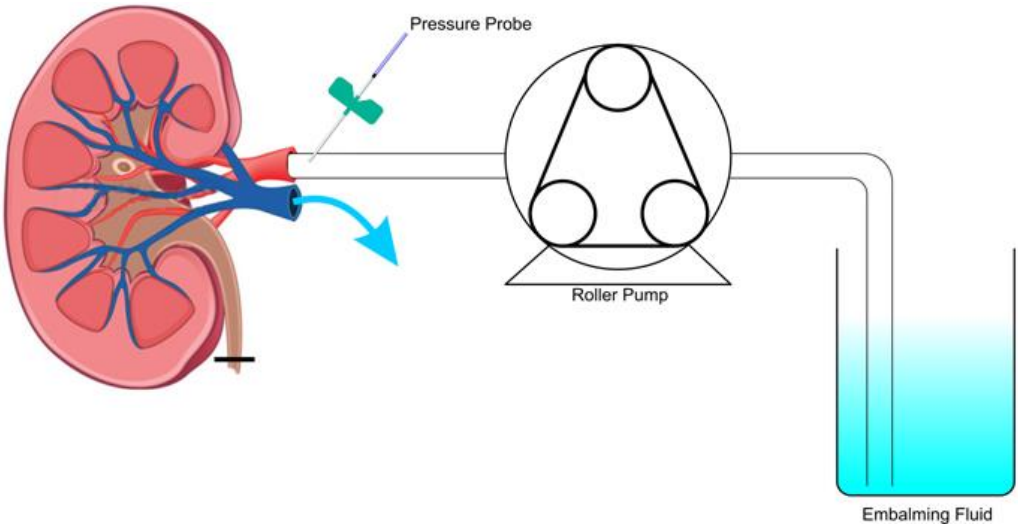

Figure 1. *Pressure-controlled embalming of pig kidneys. Thiel embalming fluid is pumped in the renal artery and a mixture of blood and/or embalming fluid eventually leaves the kidney through the renal vein.*

The embalming procedure causes diffuse parenchymatous swelling and changes the macroscopic appearance of the kidney. Next, to lower the weight, each kidney was immersed in a concentrated salt solution of 0.300 kg salt/L tap water for seven days. The amount of salt (kg) was the same as the weight of the embalmed kidney. This caused a movement of superfluous solvent molecules from the kidney into the concentrated salt solution, without interfering with the embalming procedure. After seven days, the weight, volume and appearance (*i.e.* presence or absence of shrinkage) were noted. The kidneys were then stored in a refrigerator at 9.5°C. After one week, the embalming quality was evaluated in terms of the general appearance, yeast formation and putrefaction. Fig. 2A depicts the design of the experiment schematically. Lastly, the dynamic viscosity of the administered embalming fluid was determined at 25°C with a Micro-Ubbelohde Viscometer (Schott-Geräte, Mainz, Germany).

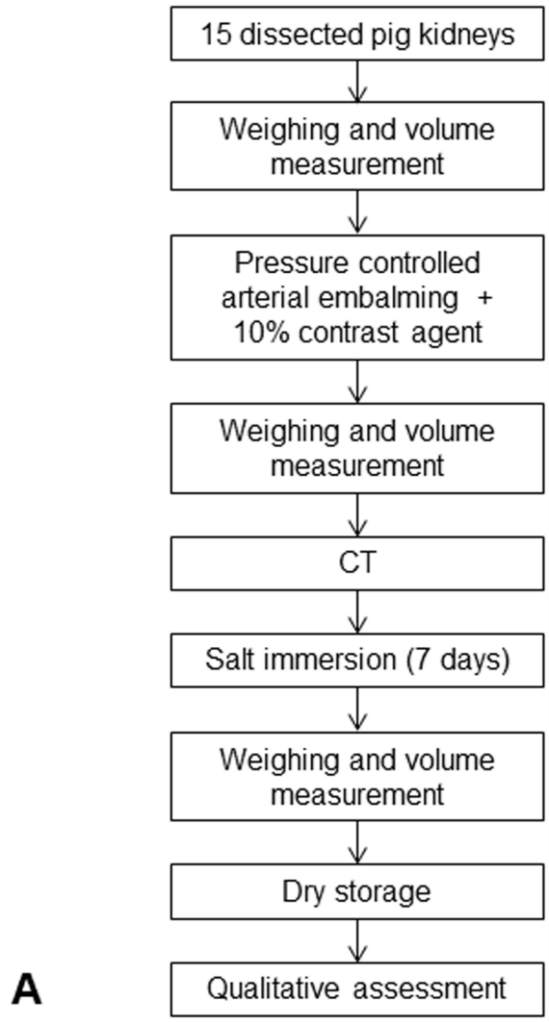

Figure 2A. *Stepwise illustration of pressure-controlled Thiel embalming followed by immersion in a concentrated salt solution.*

Experiment 2

In this experiment, a pump-driven flow was re-established in the renal vascular system. Therefore, 20 fresh pig kidneys (group 2) from the slaughterhouse underwent the same procedures described above, but no contrast agent was added during embalming (Fig. 2B). The dehydration procedure aimed to reduce the pressure on the vessels, facilitating a subsequent vascular reperfusion. The pigs were randomly divided into two groups. In one group, the vessels were reperfused with red PP (*i.e.* PP containing 43 mg/L Oil Red O [both from Sigma-Aldrich, Bornem, Belgium]). In the other group, the vessels were reperfused with diluted PEG (*i.e.* 50 % PEG 400 [Sigma-Aldrich, Bornem, Belgium] and 50 % tap water).

Before establishing the reperfusion, the volumes and weights of the kidneys were measured anew. Next, a reservoir was filled with 100 mL of the allotted perfusate. Subsequently, as

described in the first experiment, the 14-gauge arterial catheter was reconnected to a tube that was placed in the pump and the 22-gauge catheter was connected to the pressure transducer. In this way, a pump-driven, pressure-controlled (< 90 mmHg) injection of the allotted perfusate was installed to the renal artery and kidney until there was venous drainage in the reservoir. At that time, both catheters were disconnected and the renal volume and weight were redetermined. After reconnecting the catheters, a closed circulation with the perfusate was re-established, *i.e.* the venous drainage in the reservoir was pumped into the renal artery again. The procedure was interrupted after 60 and 120 minutes to measure the volumes and weights of the kidneys. After 120 minutes of reperfusion, we reassessed the renal appearance (swelling, pliability, capsular leakage and subcapsular collections).

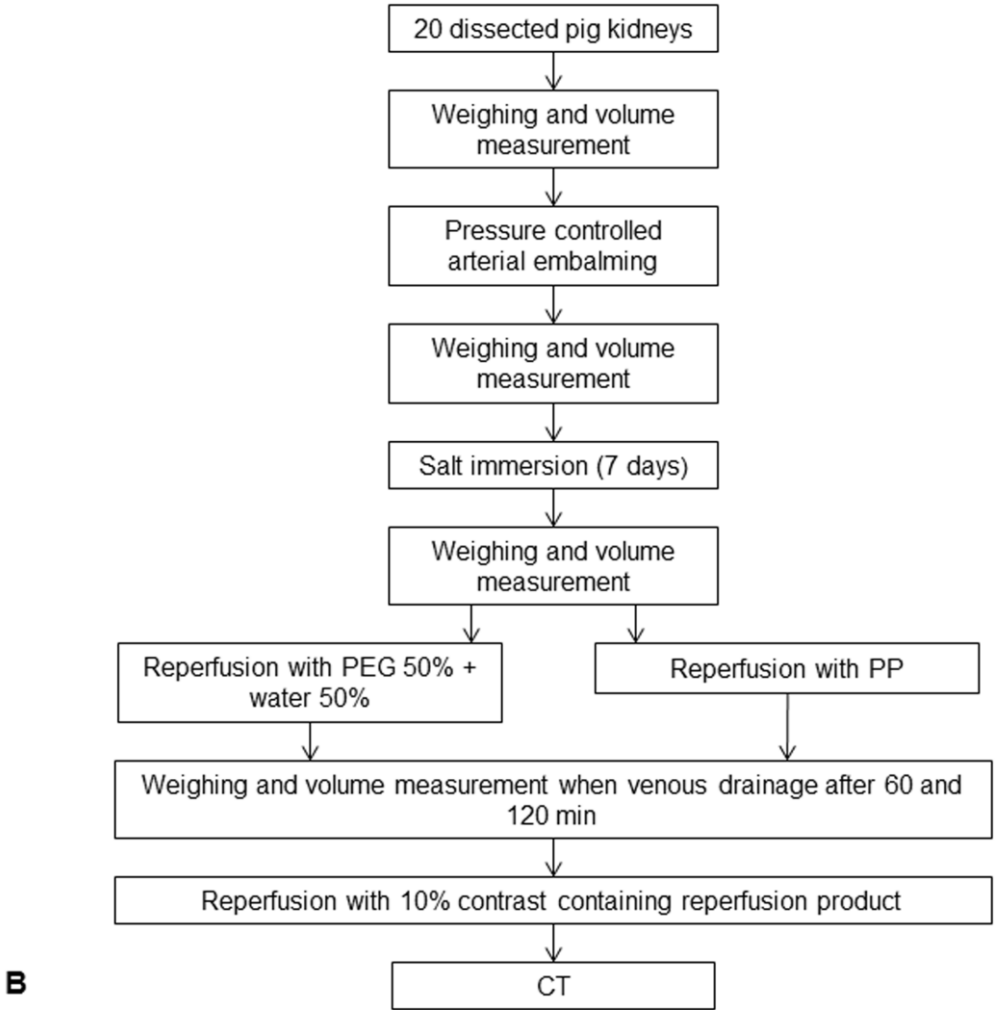

Figure 2B. *Stepwise illustration of reperfusion of Thiel embalmed and dehydrated kidneys with either PP or diluted PEG.*

Reperfusion via the artery was restarted after adding contrast agent to the remaining perfusate in the reservoir. The amount of contrast agent added was 10 % of the remaining volume of perfusate. In case of reperfusion with red PP, we used black colored Angiofil® (Fumedica AG, Muri, Switzerland). The diluted PEG was mixed with a combination of Omnipaque 300® and 2 cc of 1 % methylene blue (MB) (Sterop, Brussels, Belgium). MB was added to color the transparent diluted PEG. When the contrast containing mixture left the renal vein, the reperfusion was terminated. CT was performed to assess the vascular distribution of both perfusates. In addition, 4 specimens were frozen at -80°C and sectioned to visualize the vascular reperfusion of PP and diluted PEG.

Finally, the effects of embalming, dehydration and vascular reperfusion on tissue morphology were investigated. Therefore, cortical biopsies were taken from a fresh kidney; a Thiel embalmed kidney; a dehydrated Thiel embalmed kidney; and after 120 minutes of vascular reperfusion with PP and diluted PEG. The biopsies were stained with orcein as well as hematoxylin and eosin.

Statistical Analysis

Statistical analysis was carried out with SPSS Version 21.0. Comparisons between different groups were performed with the Friedman and Wilcoxon signed-rank test. A P value < 0.05 was deemed statistically significant.

Results

Experiment 1

Arterial administration of a fixed volume of contrast-enhanced Thiel embalming solution showed diffuse but variable renal dispersion without zones lacking contrast. In detail, the embalming product filled the renal artery and its major branches, appearing as bright white. There was an overall diffuse intermediate opacification of the renal parenchyma, with local zones of contrast accumulation. The renal surface tended to have less contrast filling, but slightly more than the renal calices. Bright opacification of the draining renal vein was often observed. The renal distribution of the embalming fluid is illustrated in Fig. 3. After embalming, the proportions of kidneys with sanguinous, serosanguinous and transparent venous drainage were 60 %, 33.3 % and 6.7 %, respectively. Embalming caused significant increases in weight (P = 0.005) and volume (P = 0.007), with on macroscopic inspection obvious swelling in 33.3 % of cases.

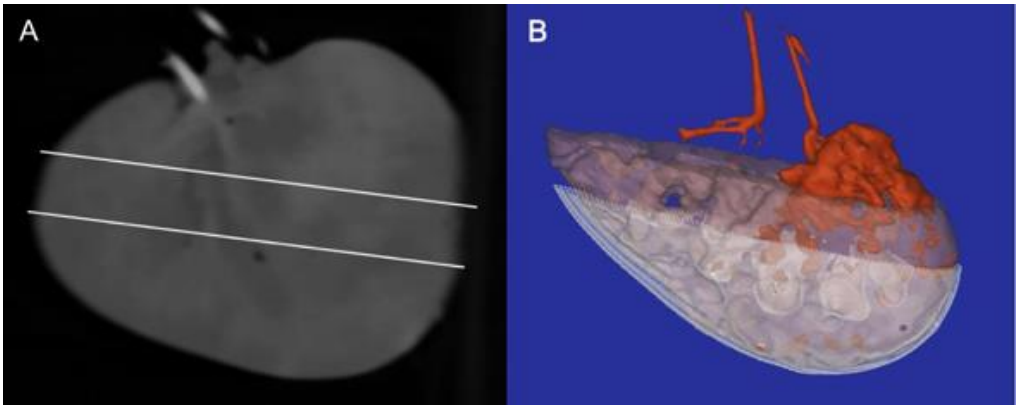

Figure. 3. *Renal spread of Thiel embalming fluid. (A) Planar CT image shows three areas of filling (contrast): bright, the main arterial and venous system and the poles-medial border of the kidney; intermediate grey, centrally distributed areas; and darker grey, areas at the core and kidney surface. Two oblique white lines represent virtual slicing through the upper and lower mid-central part of the kidney. (B) 3D representation of the same kidney; red, main arterial and venous system and one of the poles-medial border areas; transparent purple, centrally distributed areas of the kidney showing intermediate contrast filling; and white polylines, inner core areas (four cone-like structures, calices) and surface of the kidney showing the least contrast filling.*

Significant weight ($P < 0.001$) and volume loss ($P < 0.001$) were encountered due to the immersion of embalmed kidneys in concentrated salt solutions. In particular, dehydration nullified the effect of the embalming procedure. Consequently, the combination of both procedures caused mean decreases in total weight and volume of 16.1 % and 26.3 %, respectively. As a result, on inspection, the majority of kidneys were shrunken (86.7 %). Figs. 4 and 5 show how embalming and subsequent immersion in a concentrated salt solution affected weight and volume.

After one week of refrigeration, excellent preservation was observed in every kidney without yeast formation or putrefaction. The dynamic viscosity of the vascular embalming fluid was 2.17 mPa.s at 25°C.

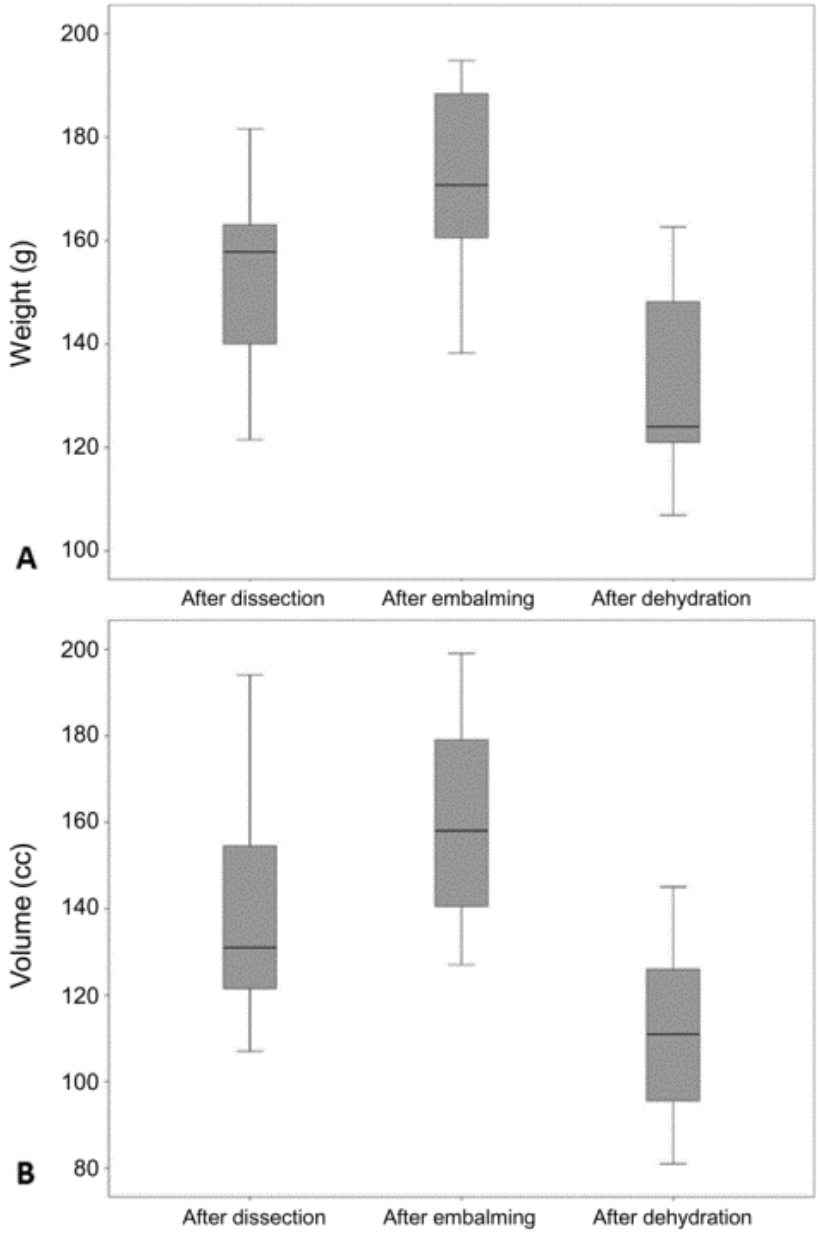

Figure 4. *Weight and volume changes in embalmed and dehydrated kidneys. Box-and-whisker plot. The bottom and top of the box are the first and third quartiles. The horizontal band in the box is the median. The horizontal ends of the lines extending vertically from the box indicate the minimum and maximum of all the data. (A) A weight gain occurs after embalming (P = 0.005) and a weight loss (P < 0.001) occurs following the subsequent dehydration, respectively. The combination of these two procedures results in a significant weight reduction (P = 0.007). (B) Swelling occurs after embalming (P = 0.007) and volume loss occurs following the immersion in a concentrated salt solution (P < 0.001). The combination of both procedures results in a significant volume reduction (P = 0.003).*

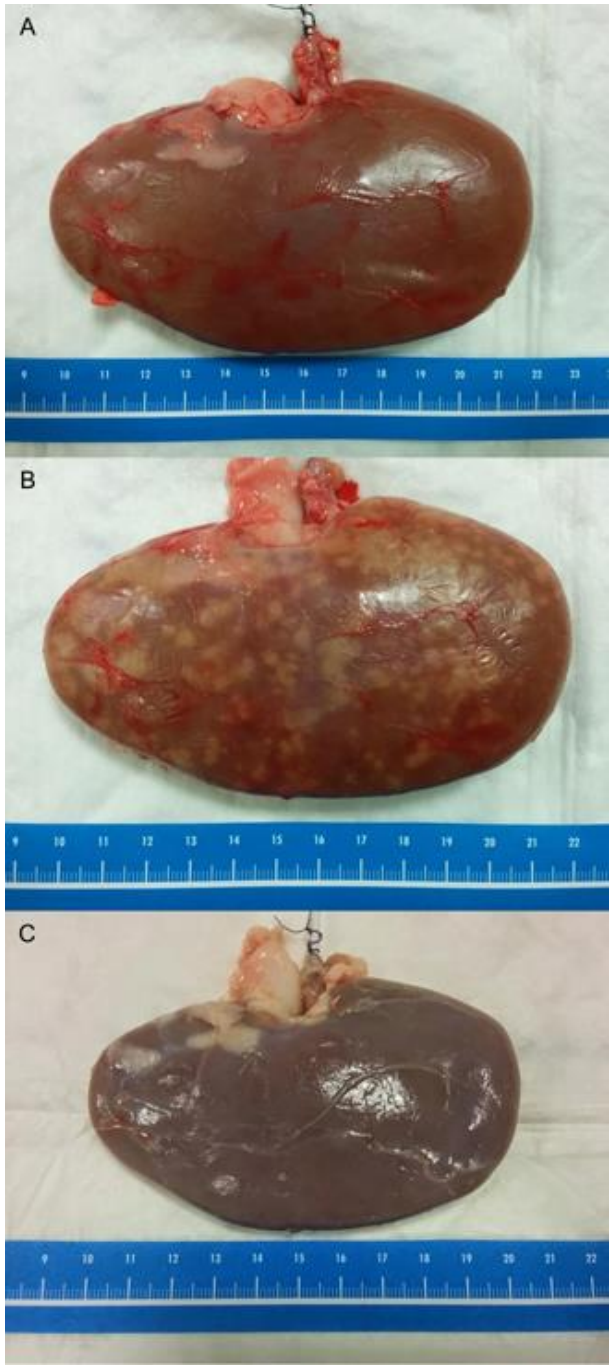

Figure 5. *Effect of embalming and dehydration on renal status. (A) Fresh pig kidney. (B) Thiel embalmed pig kidney. Note the swelling and zonal discolouring due to the embalming. (C) Thiel embalmed and dehydrated pig kidney. The organ is shrunken and discoloured, but remains pliable.*

Experiment 2

Each group contained 10 kidneys, which lost significant weight ($P < 0.0001$) and volume ($P < 0.0001$) after embalming and dehydration. In both groups, renal reperfusion for one hour caused a significant weight (both $P = 0.005$) and volume (PP: $P = 0.007$; diluted PEG: $P = 0.005$) increase. Weight ($P = 0.005$) and volume ($P = 0.032$) gain after more than 60 minutes of reperfusion with diluted PEG are substantially greater than with PP (*i.e.* $P = 0.26$; $P = 0.79$, respectively). Fig. 6 presents the weight and volume changes for the two perfusates during each step of this experiment. PP generated lower arterial pressures during the total reperfusion period ($P < 0.05$). In particular, an initial pressure decrease during the first 60 minutes ($P < 0.001$) persisted over time ($P = 0.032$). In contrast, ongoing pressure increase ($P < 0.0001$) was observed during reperfusion with diluted PEG. Pressure-time curves for both perfusates are illustrated in Fig. 7. Reperfusion of the renal vessels for 120 minutes did not affect the pliability of the organs although obvious swelling was observed in six out of ten kidneys reperfused with diluted PEG. After prolonged reperfusion with PP, two limited subcapsular collections ($n = 1$) and a few capsular drops ($n = 1$) were observed.

CT-images showed PP spread in the renal arterial tree up to the interlobular arteries without signs of extravasation. Later, drainage into the venous system occurred and clearly demonstrated PP running in the interlobar and segmental veins before eventually leaving the kidney via the renal vein. In contrast, an evenly renal distribution of diluted PEG was found making it impossible to distinguish major vessels from renal parenchyma. Fig. 8 depicts the renal distributions of PP and diluted PEG.

The embalming procedure caused cell swelling and expansion of the interstitial space. Moreover, it effectively flushed remaining red blood cells and flattened the internal elastic lamina of arterioles. Subsequent dehydration removed the excess of fluid in every structural renal component. Prolonged reperfusion with both perfusates demonstrated glomerular vessels dilation, swelling of the interstitial space and partial flattening of the arteriolar internal elastic lamina. Reperfusion with PP did not result in significant structural changes of the tubular cells. In contrast, diffuse swelling of the tubular cells was observed after reperfusion with diluted PEG. Fig. 9 illustrates the morphological changes of renal tissue during embalming, dehydration and vascular reperfusion.

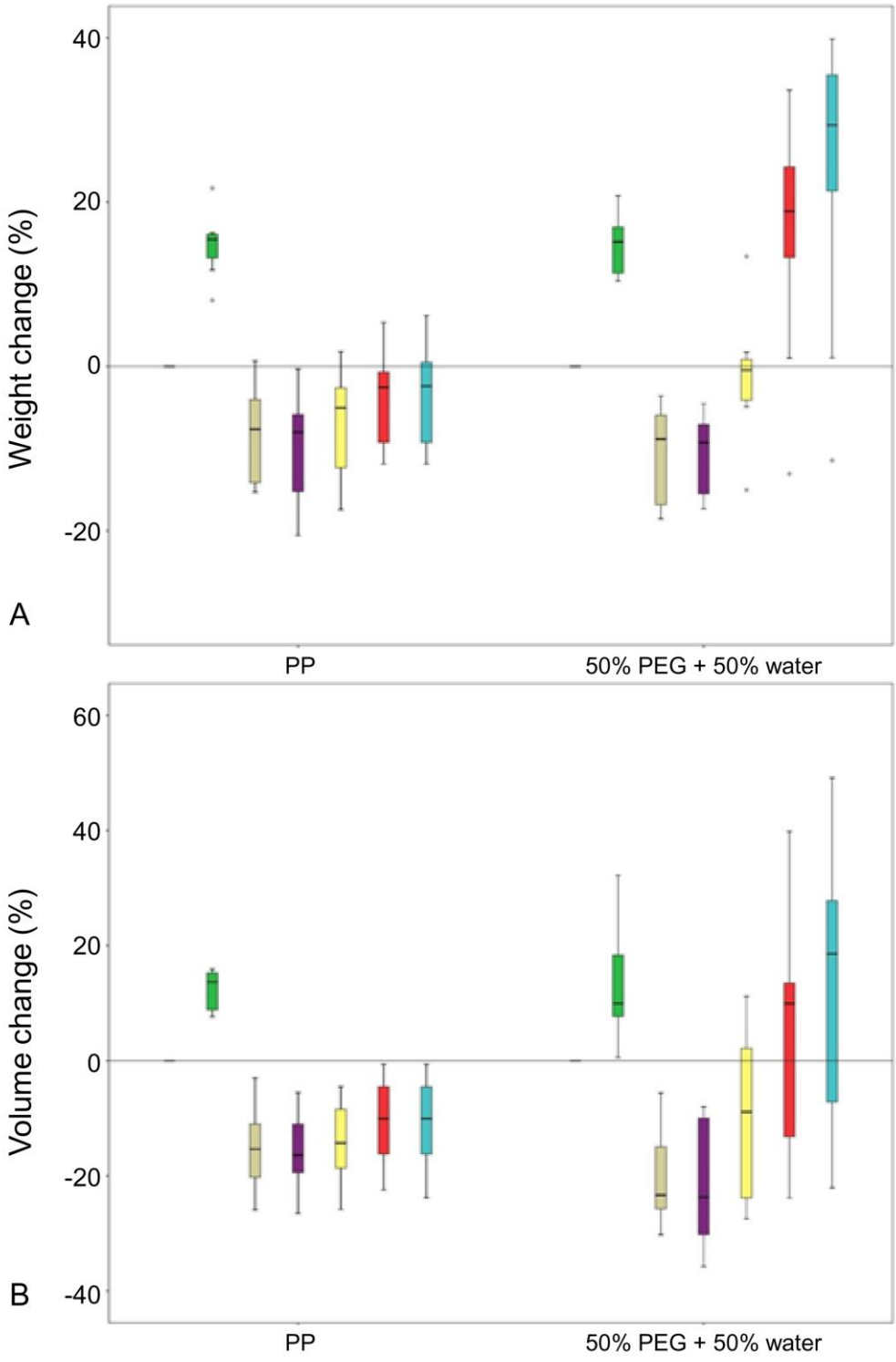

Figure 6. *Percent weight and volume change during embalming, dehydration and reperfusion of pig kidneys. Box-and-whisker plot. The bottom and top of the box represent the first and third quartiles. The horizontal band in the box is the median. The horizontal ends of the lines*

extending vertically from the box indicate the minimum and maximum of all the data. Outliers are plotted as individual points. *(A) Embalming and subsequent dehydration cause a significant weight gain (P < 0.0001) and loss (P < 0.0001), respectively. Persistent weight gain is observed during initial reperfusion with diluted PEG or PP (both P = 0.005), whereas weight gain is not present after more than 60 minutes of reperfusion with PP (P = 0.26). In contrast, ongoing weight gain (P = 0.005) is noted during reperfusion for more than 60 minutes with diluted PEG. (B) Embalming and subsequent dehydration cause a significant volume increase (P < 0.0001) and loss (P < 0.0001), respectively. In the beginning, a continuous volume increase is noted in the case of reperfusion with diluted PEG (P = 0.005) as well as PP (P = 0.007). No further increase is observed after more than 60 minutes of reperfusion with PP (P = 0.79), whereas an ongoing volume gain is present in the case of diluted PEG (P = 0.032). Weight/volume after embalming = green; after dehydration = brown; at the start of reperfusion = purple; at first venous drainage = yellow; after 60 minutes of reperfusion = red; after 120 minutes of reperfusion = blue.*

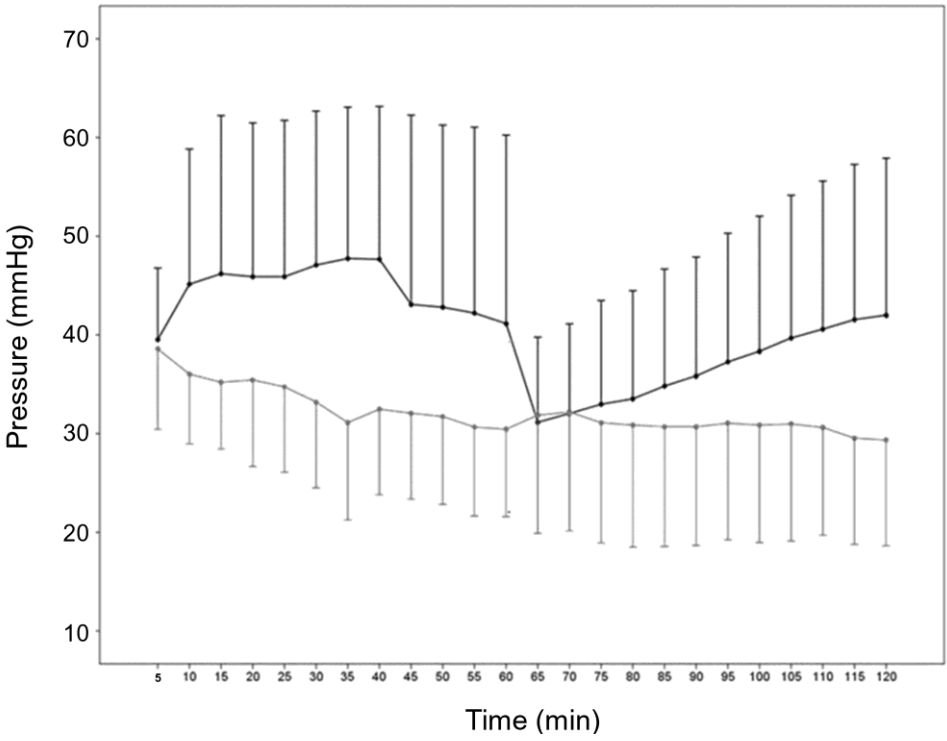

Figure 7. Arterial pressure during renal reperfusion. *PP generates lower pressures than diluted PEG (P < 0.05). Ongoing pressure decrease is observed during more than 60 minutes of reperfusion with PP (P = 0.032), whereas the pressure increases further (P < 0.0001) when diluted PEG is used. The generated pressures remain, however, lower than the mean arterial blood pressures in adult pigs. The error bars with 95 % confidence intervals are shown.*

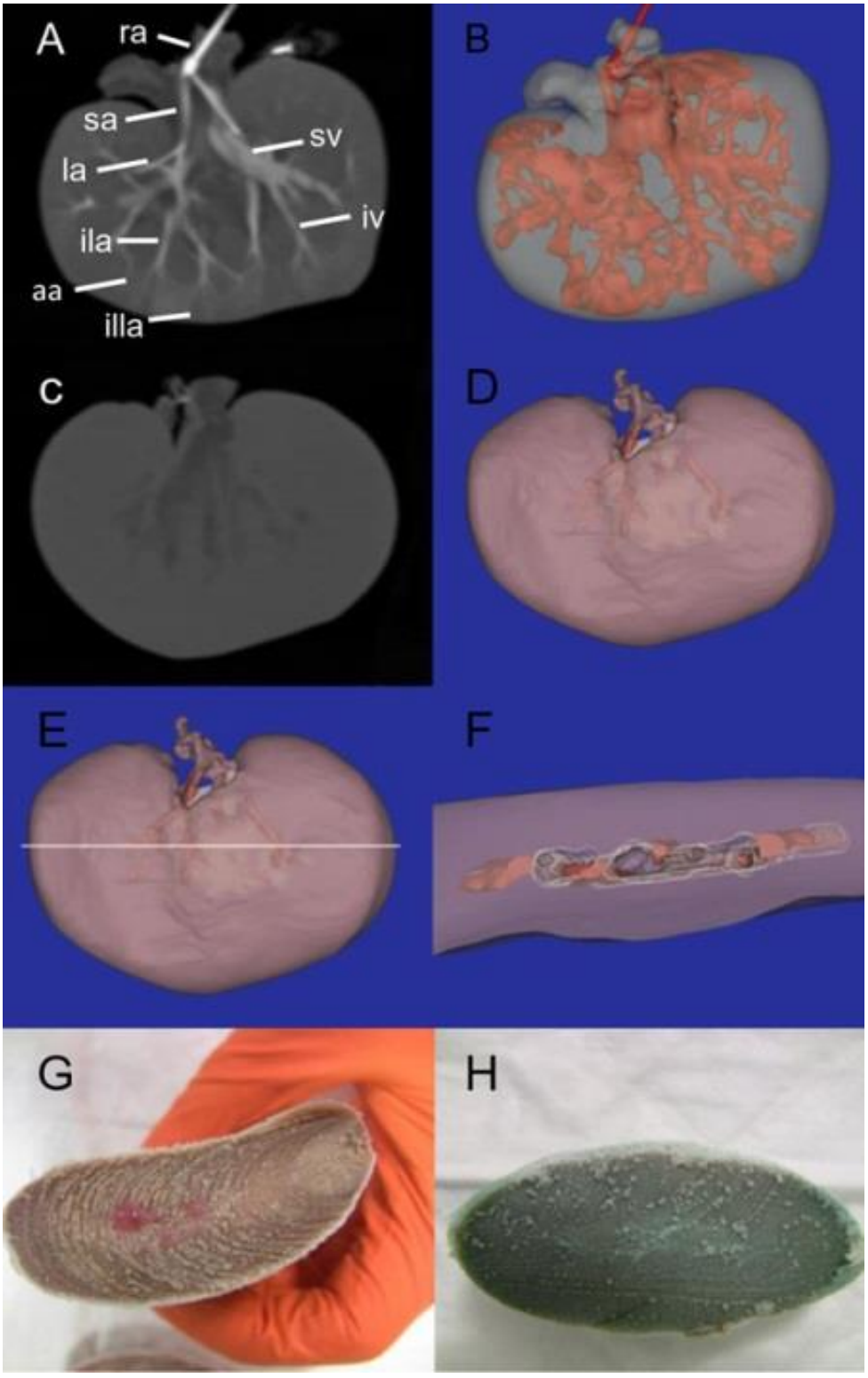
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Figure 8. *Contrast-enhanced reperfusion of Thiel embalmed and dehydrated pig kidneys with PP and diluted PEG. (A) Planar CT image shows two distinct areas of filling: the arterial and venous system (bright) demonstrating that PP recruits the major renal vessels and the renal tissue (dark grey). ra, renal artery; sa, segmental artery; la, lobar artery; ila, interlobar artery; aa, arcuate artery; illa, interlobular artery; iv, interlobar vein; sv, segmental vein. (B) 3D representation of the same kidney; red, arterial and venous system; transparent blue-grey, renal tissue showing less contrast. (C) Planar CT image of reperfusion with diluted PEG illustrates one uniform area of filling: the arterial and venous system together with the renal tissue (light grey) and centrally a darker area (dark grey) representing the renal calices. (D) 3D representation of the same kidney. The renal tissue is transparent purple and the segmental vessels are highlighted in red for illustration purposes. The central white polylined structure depicts the renal calices. (E) The white horizontal line represents the cross-sectional slice through the mid-central part of the same kidney containing renal tissue, segmental vessels and the renal calices. (F) Top view of the lower part following the virtual slicing illustrated in E. Transparent purple, renal tissue; the segmental vessels are highlighted in red and have the same contrast as the renal tissue; white polylines border the calices, which are not filled or are less filled by the contrast fluid. (G) Cross-section through frozen Thiel embalmed pig kidney reperfused with PP. Red PP is present in the major renal vessels. (H) Cross-section through frozen Thiel embalmed pig kidney reperfused with diluted PEG. Blue diluted PEG diffusely stains the sectioned renal surface.*

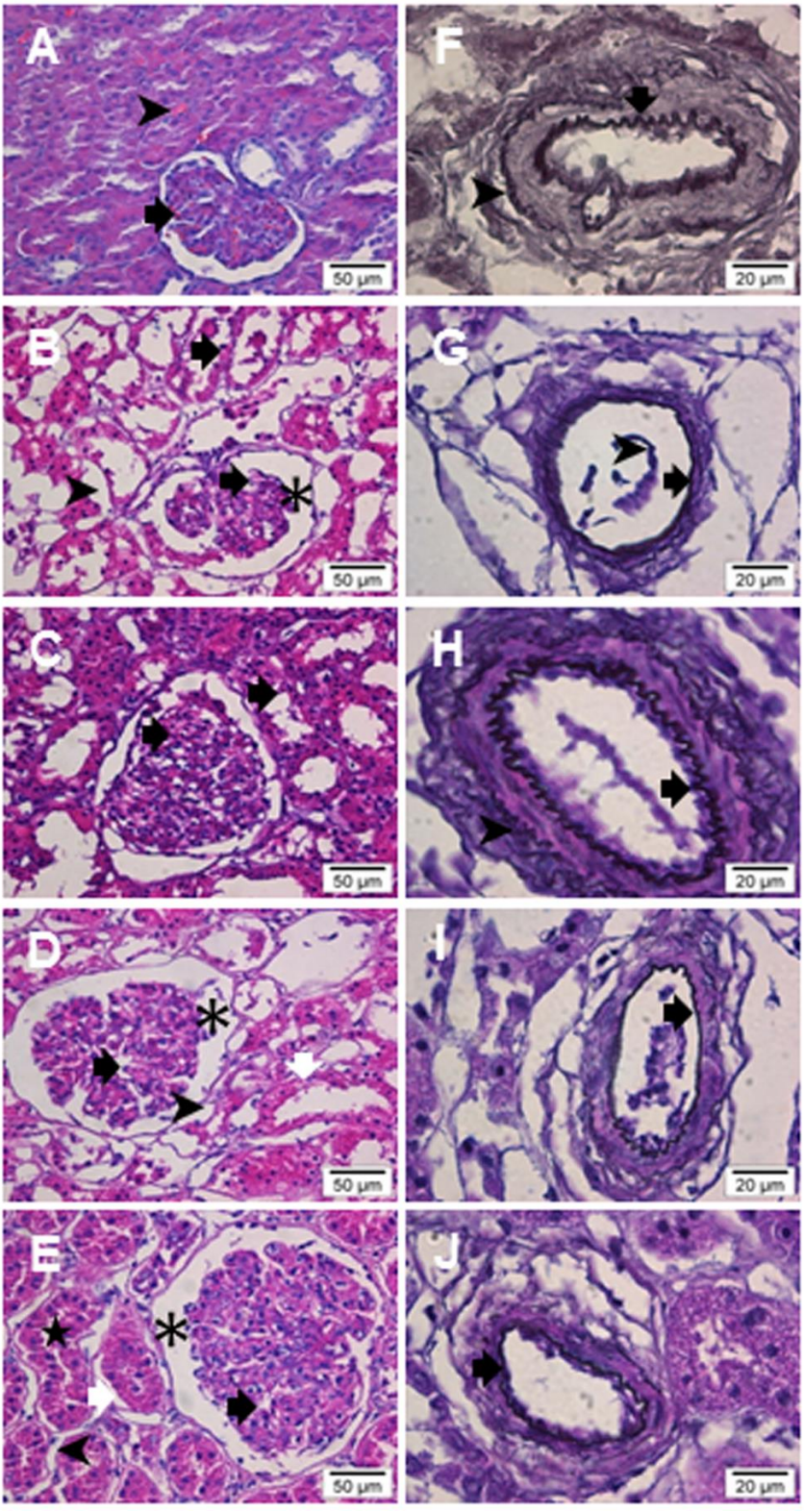
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Figure 9. *Morphological changes of renal cortex during embalming, dehydration and vascular reperfusion. Pig cadaver kidneys. A-E: Hematoxylin and Eosin staining (magnification: x 400). (A) Fresh renal cortex. Glomerular vessels (black arrow) and peritubular capillaries (arrowhead) contain red blood cells. (B) Thiel embalmed renal cortex. Cloudy cell swelling causes rupture of cells (black arrows) and glomerular disintegration. Embalming fluid accumulates in the interstitium (arrowhead) and Bowman's space (asterisk). Red blood cells are absent. (C) Dehydrated Thiel embalmed renal cortex. Renal histology is similar to the fresh status. The glomeruli have a compact structure and cells have a dens appearance but their shape remains irregular (black arrows). There is no expansion of the interstitial space. (D) Reperfusion of Thiel embalmed dehydrated renal cortex with PP. Reperfusion causes dilation of the glomerular vessels (black arrow) and expansion of Bowman's space (asterisk) and interstitial space (arrowhead). Tubular cells remain irregular and flat (white arrow) as in the dehydrated status suggesting that the perfusate does not interact with these cells. (e) Reperfusion of Thiel embalmed dehydrated renal cortex with diluted PEG. Glomerular vessels (arrow) and Bowman's space (asterisk) are dilated. There is a fluid shift into the interstitial space (arrowhead). Widening of subepithelial space (white arrow) of enlarged tubular cells presumably due to uptake of the water component of diluted PEG. This causes narrowing of tubular lumina (star). F-J: Orcein staining (magnification: x 1000). (F) Fresh renal cortex. The wavy internal elastic lamina of arterioles is deep red brown (black arrow). The tunica adventitia is lightly stained (arrowhead). (G) Thiel embalmed renal cortex. More than half of the internal elastic lamina is flattened suggesting fluid accumulation in the vessel wall (black arrow). Partly detached endothelial cells (arrowhead). (H) Dehydrated Thiel embalmed renal cortex. Return of the wavy appearance of the internal (black arrow) and external (arrowhead) elastic membranes due to fluid loss. (I) Reperfusion of Thiel embalmed dehydrated renal cortex with PP. Partial flattening of the internal elastic lamina probably due to an increase of perfusate in the vessel wall (black arrow). (J) Reperfusion of Thiel embalmed dehydrated renal cortex with diluted PEG. The internal elastic lamina lost parts of its scalloped appearance which may be caused by a build-up of diluted PEG in the wall (black arrow).*

Discussion

This study was the first to assess the vascular spread of contrast-enhanced Thiel embalming solution and to determine its effect on the appearance of the original fresh tissue. We demonstrate that pig kidneys are a suitable model to evaluate this technique. Indeed, CT imaging showed that when a volume was injected as determined by Thiel (*i.e.* comparable to 18.170 kg for a human cadaver weighing 80 kg), there was a widespread distribution in the kidney without filling defects.

This distribution is feasible when embalming is done under controlled arterial pressure using a pump. We assume that a pressure lower than 90 mmHg is beneficial to limit unnecessary vessel damage and ongoing local congestion of embalming product in the interstitial space. The mean arterial blood pressure in adult pigs is 90 mmHg. Despite controlling the pressure, local accumulation, interstitial space swelling, partial flattening of the arteriolar internal elastic membrane and significant increases in weight and volume were observed. These changes are likely due to the injection of too much embalming fluid, which eventually extravasates and they are favored by the low viscosity of the embalming solution. Note that the embalming fluid must perfuse the capillaries for effective preservation. Due to early postmortem autolysis, leakage into the interstitial space is probably unavoidable but must be minimized to avoid swelling and deformation. Thiel embalming fluid is very thin and is less viscous than blood (*i.e.* 4–5 mPa.s at

body temperature) and therefore, it easily flows through the smallest vessels without generating high inlet pressures.[89] The use of the large volume of vascular embalming solution, as originally proposed by Thiel, must be questioned as it causes significant increases in weight and volume. During embalming, venous loss of this solution (*i.e.* transparent or serosanguinous venous drainage) was observed in 40 % of the kidneys, which were, however, adequately preserved. It should be borne in mind that diminishing the embalming volume without hampering tissue preservation must be undertaken with caution. Consequently, dehydration of embalmed kidneys by salt water immersion can be a good solution. Notably, blood is still present in the vast majority of embalmed kidneys, suggesting that complete venous drainage of blood is superfluous. Notwithstanding its exploratory character, this study observed the course of the embalming fluid in intact vessels, making it impossible to assess if atherosclerosis affects the notable variation in the quality of embalmed tissue within and among Thiel cadavers (unpublished data). Other causes for this observation may be the varying periods between death and the initiation of embalming or deficiencies in the embalming properties of the Thiel fluid, which are unlikely.

Intriguingly, vascular perfusion alone effectively embalmed every kidney, enabling storage in the refrigerator for more than two months. This result could be expected because the CT images revealed no zones lacking embalming fluid. It is important to note that we did not immerse the kidneys in an embalming bath as recommended by Thiel. As extensively demonstrated by Thiel, immersion is essential to embalm the skin and subcutaneous tissue of human bodies, but immersion seems superfluous when preserving a kidney. As mentioned above, embalming causes swelling and deformation, so an appropriate and simple method was examined to re-establish the original weight of the embalmed kidneys in a fast, controlled manner. Embalming-induced weight and volume gains can be successfully reduced by immersion in a concentrated salt solution. Shrinkage of the interstitial space and return of the original wavy structure of the internal and external elastic membranes illustrate this fluid loss. This was the first report showing that Thiel embalmed organs can be quickly dehydrated under controlled circumstances to reinstate their original status. Note that the majority of kidneys are obviously shrunken and discolored due to blood loss but remain pliable.

Our intention to reduce the weight of embalmed tissue is part of a wider project on blood-like vascular reperfusion of Thiel embalmed human cadavers. Therefore, a continuous pump-driven flow mimicking blood circulation was tested in the vessels of dehydrated Thiel embalmed kidneys. We clearly demonstrate that both PP and diluted PEG effectively circulate in this kidney model. CT imaging confirms they had a widespread renal distribution without filling defects. The results indicated that PP clearly fills the major vessels and renal tissue, whereas diluted PEG more diffusely spreads in the kidney. Certainly, PP is superior because no significant weight or volume gain was observed after more than 60 minutes of reperfusion.

Moreover, PP generates lower pressures and does not influence the pliability and appearance of the organs. Indeed, there is some expansion of the interstitial space and partial flattening of the internal elastic lamina of the arterioles suggesting extravasation, but the major reason why lower pressures are observed is the absence of structural tubular changes during reperfusion with PP. As PP is osmotically inactive, it does not interfere with the tubular cells, which remain flat. In contrast, we observed ongoing weight and volume gain and pressure increase during prolonged reperfusion with diluted PEG. This observation can be explained by cellular uptake of the water component causing diffuse swelling of the tubules across the renal parenchyma. Note that we encountered a gradual loss of fluid from the organ into the environment during storage. Probably, this is the water component of the diluted PEG, which leaves the kidney and may permit a second reperfusion. Researchers previously reperfused animal models with several types of perfusates.[87, 192, 195] However, these experiments were performed on fresh tissue.

Hence, it is likely that combining embalming and dehydration will enable reperfusion of Thiel embalmed human cadavers. In our experience, embalming-induced swelling without subsequent dehydration hindered pump-driven vascular reperfusion in an adult pig (unpublished data). Interestingly, a long immersion of a Thiel embalmed pig in a concentrated salt solution caused significant weight loss (unpublished data), which may allow for vascular reperfusion with PP. Thus, future research should focus on validating this model in an embalmed and dehydrated organ system or total body.

Conclusions

The Thiel embalming procedure is a complex process and several essential steps must be fulfilled for adequate preservation. We show that Thiel embalming fluid has a low viscosity and therefore easily flows in intact vessels and diffusely spreads in a kidney model. As such, other factors may contribute to the observed variation in tissue preservation within and among Thiel embalmed cadavers. Further, it is recommended that this mixture is administered under physiological pressure using a pump to limit vascular damage and needless subsequent extravasation that later escapes from the body. We posit that injecting a larger volume to enhance the embalming quality will result in unnecessary swelling and deformation and we underscore that the recommended embalming volume is sufficient, but useless congestion occurs. Immersion in a concentrated salt solution is a quick, easy and controlled method to remove accumulated fluid and re-establish the organs' original status without jeopardizing the embalming quality. The present study provides a useful basis for assessing the course of the embalming mixture in human bodies, for restoring the cadavers' initial appearance and for broadening our knowledge about the embalming procedure. Furthermore, this model seems ideal to establish prolonged vascular reperfusion in Thiel embalmed cadavers by mimicking lifelike circumstances to learn human anatomy, to test new devices and to practice surgical procedures.

PART E

Long dynamic Thiel embalmed pig reperfusion, evaluation of the microscopic vessel recruitment and feasibility for surgical practice. A plea for pump-driven embalming.

Chapter 9

Thiel embalming and creation of lifelike reperfusion from artery to vein

W.Willaert, F. Tozzi, T. Van Hoof, P.Pattyn, K D'Herde

Eur Surg Res. 2015; In revision

Abstract

Background Vascular reperfusion of Thiel cadavers can aid surgical and anatomical instruction. This study investigated whether ideal embalming circumstances provide lifelike vascular flow, enabling surgical practice and enhancing anatomical reality.

Methods Pressure-controlled pump-driven administration of blue embalming solution was assessed directly postmortem in a pig model (n = 4) in terms of perfusion parameters, intracorporeal spread and embalming quality. Investigation of subsequent pump-driven vascular injection of PP included assessment of flow parameters, intracorporeal distribution, anatomical alterations and feasibility for surgical training. The distribution of PP was analyzed in pump-embalmed pig and gravity-embalmed human small intestines.

Results Embalming lasted 50–105 min and maximum arterial pressure was 65 mmHg. During embalming, the following consecutive alterations were observed: arterial filling, organ coloration, venous perfusion and further tissue coloration during the next weeks. Most organs were adequately preserved. PP generated low arterial pressures (<30 mmHg) and drained through the venous cannula. Generally, realistic reperfusion and preservation of original anatomy were observed, but leakage in the pleural, abdominal and retroperitoneal cavities occurred and computed tomography showed edematous spleen and liver. Reduction of arterial flow rates after venous drainage is prerequisite to prevent anatomical deformation, allowing simulation of various surgeries. In pump-embalmed pig small intestines, PP flowed from artery to vein through the capillaries without extravasation. In contrast, arterioles were blocked in gravity-embalmed human tissues.

Conclusion In a pig model, immediate postmortem pressure-controlled pump embalming generates ideal circumstances for (micro)vascular reperfusion with PP, permitting lifelike anatomy instruction and surgical training.

Introduction

The last two decades have seen the wide expansion of minimally invasive surgical techniques, which have long learning curves and require fine skills and confident use to avoid perioperative complications and guarantee patient safety.[187, 196-199] As a result, surgical training models have been developed. VT and VR simulators can improve expertise in the surgical field.[163, 200] Animal models involving pigs are valuable, but their anatomy does not adequately resemble that of humans.[201]

Human cadavers with reperfused vessels provide a more suitable model system. Many researchers have reported on the use of reperfused body parts for surgical training.[30, 94, 96, 170-173, 175] Garrett published the first report of pump-driven arterial–arterial circulations in more than 200 complete fresh cadavers.[97] Despite the merits of this technique, edema and lack of venous circulation were important limitations. In 2010, researchers simulated arterial pulsations and installed static venous pressure in 11 complete fresh cadavers.[96] However, a real flow was absent and the arterial and venous circuits were separated. Similar training models were developed subsequently.[91-94] Recently, arteriovenous shunting provided real flow in 14 formalin-fixed partial and complete cadavers.[95] This model was used for trauma training, but bleeding and tissue consistency were not lifelike.

These models lack prolonged physiological flow from artery to vein. Thiel-embalmed tissues, characterized by soft preservation, are ideal for the establishment of vascular reperfusion. Long lifelike reperfusion from artery to vein without significant weight or volume gain has been established in Thiel-embalmed pig kidneys using PP **(chapter 8)**.[202] Researchers have also reperfused the arterial pedicles of several flaps in six Thiel-embalmed cadavers.[59] This valuable surgical training model, however, had weak venous return due to the use of tap water.

Prolonged reperfusion of Thiel-embalmed human cadavers from artery to vein has not yet been performed. The success of this technique may depend on several factors, including the technique used to inject embalming solution into the vessels. Currently, we administer the solution by gravity and often encounter insufficient preservation, which may hamper subsequent vascular reperfusion. A more controlled technique of embalming solution administration (*i.e.* by pumping) is needed.

Thus, the first aim of this study was to establish ideal circumstances for Thiel embalming with the use of a pump in a pig model and to evaluate the procedure in terms of flow parameters, embalming solution distribution and embalming quality. Second, in the same model, we assessed the gross anatomical effects and feasibility for surgical training of long reperfusion from artery to vein with PP. Third, we investigated the microvascular flow of PP in pump-embalmed pig small intestines and compared with that obtained in gravity-embalmed human tissues.

Materials and Methods

The human and animal ethics committees of Ghent University Hospital, Belgium, approved this research (approval codes 2010/648 and 11/36, respectively), which was performed conform to institutional standards.

Cannulation of the pig model

Three anesthetized 16-week-old pigs were euthanized by intravenous administration of T61 solution (MSD Animal Health, Brussels, Belgium). Immediately after euthanasia, the first pig was split cranially from the diaphragm muscle for lower-body embalming. Cannulas were inserted and affixed in the abdominal aorta (18F; Maquet, Hirrlingen, Germany) and caudal vena cava (24F; Maquet) in the cranial–caudal direction. The second pig was split and cannulated identically, but tubes were also placed in the thoracic aorta (20F; Maquet) and right atrium (28F; Maquet) in the caudal–cranial direction to embalm the upper body. In both cases, bone wax was inserted into the spinal canal to avoid leakage during embalming. To embalm the whole body of the third pig, a lateral cervical incision was made and T-tubes were placed in the common carotid artery (10F; Bard Medical, Olen, Belgium) and external jugular vein (20F; Bard Medical). Specimens were then weighed.

Pump-driven embalming and dehydration

The arterial cannula was connected to a tube, which was placed in a roller pump (Watson-Marlow 520 U, Zwijnaarde, Belgium) for injection of the Thiel embalming mixture. The weight of the embalming solution was 22.7 % of each specimen's weight, in accordance with Thiel's proposed administration of 18.170 kg embalming solution to an 80-kg human cadaver.[32] Fifty milliliters of 1 % MB (Sterop, Brussels, Belgium) were mixed with the embalming solution, enabling visualization of its flow in the specimens. Laparotomy was then performed and sternotomy was also done in the third pig. After the initiation of embalming, the cannulated artery was punctured with a BD Insyte-W 22-gauge catheter (BD Vialon, Madrid, Spain) and connected to an ultraminiature fiber-optic pressure transducer (Samba 201 CAP; Harvard Apparatus, Les Ulis, France). The maximum arterial pressure allowed was 90 mmHg, in agreement with the *in vivo* mean arterial blood pressure of pigs. Embalming volume, maximum flow rate and embalming procedure duration were noted. Figure 1 illustrates the vascular embalming procedure in a total pig.

Vascular embalming was followed by weighing and immersion in a Thiel bath for 7 days to embalm the skin and subcutaneous tissue. Embalming obviously causes weight gain, which may hinder subsequent vascular reperfusion. Therefore, after weighing, specimens were immersed in a concentrated salt solution (0.300 kg salt/L tap water) for 7 days to induce osmotic dehydration. The quantity of salt was equivalent to the embalmed specimen's weight. Willaert et al. previously observed substantial weight loss with this method in Thiel-embalmed

pig kidneys **(chapter 8)**.[202] Next, the pigs were weighed and spread of embalming product in the tissues was reassessed. The specimens were stored for several weeks, during which the quality of embalming was monitored in terms of mold formation and putrefaction.

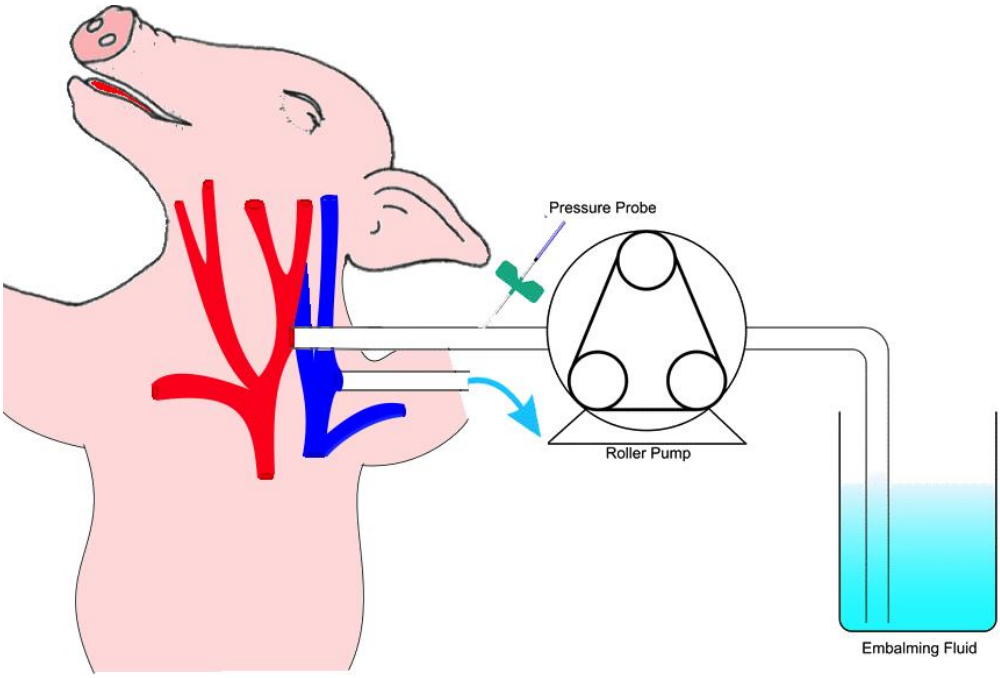

Figure 1. *Pressure-controlled embalming of a whole pig. Blue colored Thiel embalming fluid is pumped in the common carotid artery (black arrow). A catheter is inserted in the arterial cannula for pressure measurement. Clots leave the body through the external jugular vein (blue arrow).*

Vascular reperfusion and testing surgical procedures

Pump-driven, pressure-controlled (<65 mmHg) injection of red PP (*i.e.* PP with 43 mg/L Oil Red O, both from Sigma-Aldrich, Bornem, Belgium) mixed with 6 % contrast agent Angiofil® was established in the arterial tube. The vascular flow of PP in the thoracoabdominal organs and tissues was inspected and its gross anatomical effects were observed. Total reperfusion time, flow rate, maximum arterial pressure and time until venous drainage of PP were noted. The specimens were transferred to a CT scanner (Somatom Definition Flash; Siemens Healthcare Sector, Forchheim, Germany) after 80 min to visualize the thoracic, abdominal and peripheral flow of PP. Flow was then re-established for several hours, permitting the performance of surgical procedures and techniques in realistic circumstances: skin incision and hemostatic control of cut subcuticular vessels; dissection of major vessels and division of side branches with ligatures or hemostatic clips; performance and quality assessment (e.g. leakage and stenosis) of vascular anastomoses; suturing to control parenchymatous bleeding during partial

kidney and liver resection; isolation and division of hilar vessels during splenectomy and nephrectomy; and control of hemorrhages of transected intestinal walls.

Microvascular reperfusion of pump-embalmed pig small intestines

To examine the microvascular flow of PP, a segment of pig small intestines was obtained from the slaughterhouse. Within 2 hours postmortem, the feeding artery was cannulated with an iron catheter. The catheter was connected to a tube placed in the roller pump. As described above, the artery was also punctured with a catheter for pressure measurement. The pump then injected blue Thiel embalming solution into the artery at a flow rate of 3 mL/min. The weight of the embalming solution was 22.7 % of the specimen's weight. After embalming, the mesenteric peritoneum was removed locally to permit vessel inspection. The small intestine was placed on the stage of a modified Olympus BX51WI microscope (Olympus NV, Aartselaar, Belgium), where a pump-driven flow of red PP was initiated at a rate of 0.6 mL/min. The specimen was examined by light microscopy and static images of the microcirculation were captured in real time using Cell B image software (Olympus NV). A lipophilic carbocyanine dye, 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI), that incorporates into endothelial cell membranes upon contact, was employed to visualize the microvascular flow of PP.[203] For this procedure, 1 mg DiI was dissolved in 0.2 mL 99.8 % ethanol and mixed with 1.8 mL red PP. During ongoing flow, 0.5 mL of this mixture was added as a bolus. The specimen was examined under fluorescence microscopy with an HBO 50W mercury lamp (Osram, Zaventem, Belgium) and a filter set (excitation filter: 330–385 nm) to detect the mixture. Static and dynamic images of the microcirculation were obtained. Digital images were captured in real time using a high-sensitivity digital camera (model C8484-05; Hamamatsu Photonics, Hamamatsu, Japan). As a control, reperfusion was established in the same manner in a segment of small intestine prelevated from the third Thiel-embalmed pig.

Microvascular reperfusion of gravity-embalmed human small intestines

The intestinal flow of PP was analyzed in isolated small intestines prelevated from two Thiel-embalmed human cadavers. Unlike the procedure used for pig intestines, embalming was done by gravity. The flow rate of PP was 2 mL/min.

Results

Pump-driven embalming and dehydration

Directly after euthanasia, Thiel embalming solution flowed at gradually increased rates without exceeding an arterial pressure of 65 mmHg. Embalming time was 50–105 min and caused venous drainage of clots in all cases. The embalming parameters are presented in Table 1. Mean embalming-induced weight gain was 17.95 %. Immersion in a Thiel bath and concentrated salt solution resulted in mean weight losses of 3.0 % and 2.9 %, respectively.

At the beginning of the vascular embalming procedure, blue embalming solution was noticed in arterial branches to the stomach, small intestine, lungs and intercostal muscles. Then, organs exhibited blue spots (*e.g.* heart and eyes) or a bluish color (*e.g.* small intestine). Later, the product left the organs and entered the veins. Two weeks after vascular embalming, further blue coloration of tissues (*e.g.* skin and muscles) and organs (*e.g.* kidneys and heart) was observed in all specimens. Some organs (*e.g.* liver and spleen), however, exhibited no blue coloration. Figure 2 illustrates the distribution of the embalming solution. All organs and tissues except the pancreas and greater omentum (which were brownish) were preserved adequately. During prolonged storage, fungal skin infection developed on some distal legs.

Table 1: *Parameters during vascular embalming and subsequent reperfusion with PP. The maximum flow rate during embalming varied, but the arterial pressure was not above 65 mmHg. The pump injected the embalming solution in a fast and controlled way. In every specimen, the embalming mixture eventually entered the veins and flushed clots through the venous cannula. PP generated low arterial pressures during vascular reperfusion until venous drainage. In lower body 1, high flow rates remained after venous outflow was established, causing predominant accumulation of PP in the small intestinal wall, mesentery and retroperitoneal space. This phenomenon was not observed in the other cases where lower flow rates were installed after venous outflow occurred, allowing to perform surgical procedures.*

	Vascular embalming				Reperfusion with PP				
Case no.	Maximum flow rate (mL/min)	Maximum arterial pressure (mmHg)	Duration (min)	Volume (mL)	Maximum flow rate until venous drainage (mL/min)	Maximum arterial pressure until venous drainage (mmHg)	Time until venous drainage (min)	Flow rate after venous drainage (mL/min)	Total reperfusion time (min)
Lower body 1	50	65	105	2,750	45	30	52	40	82
Lower body 2	60	45	50	2,500	5	30	80	1 to 5	320
Upper body	30	65	80	1,640	20	24	12	1 to 25	320
Total body	75	54	90	4,000	9	25	90	4	180

Vascular reperfusion and testing surgical procedures

A maximum arterial pressure of 30 mmHg was measured in all specimens, despite variation in flow rates until the drainage of PP in the venous cannula (Table 1). In the lower body of the first pig, a high flow rate (40 mL/min) persisted after venous drainage, resulting in accumulation of PP in the small intestinal wall, mesentery and retroperitoneal space. This accumulation caused tissue deformation and prevented surgical training.

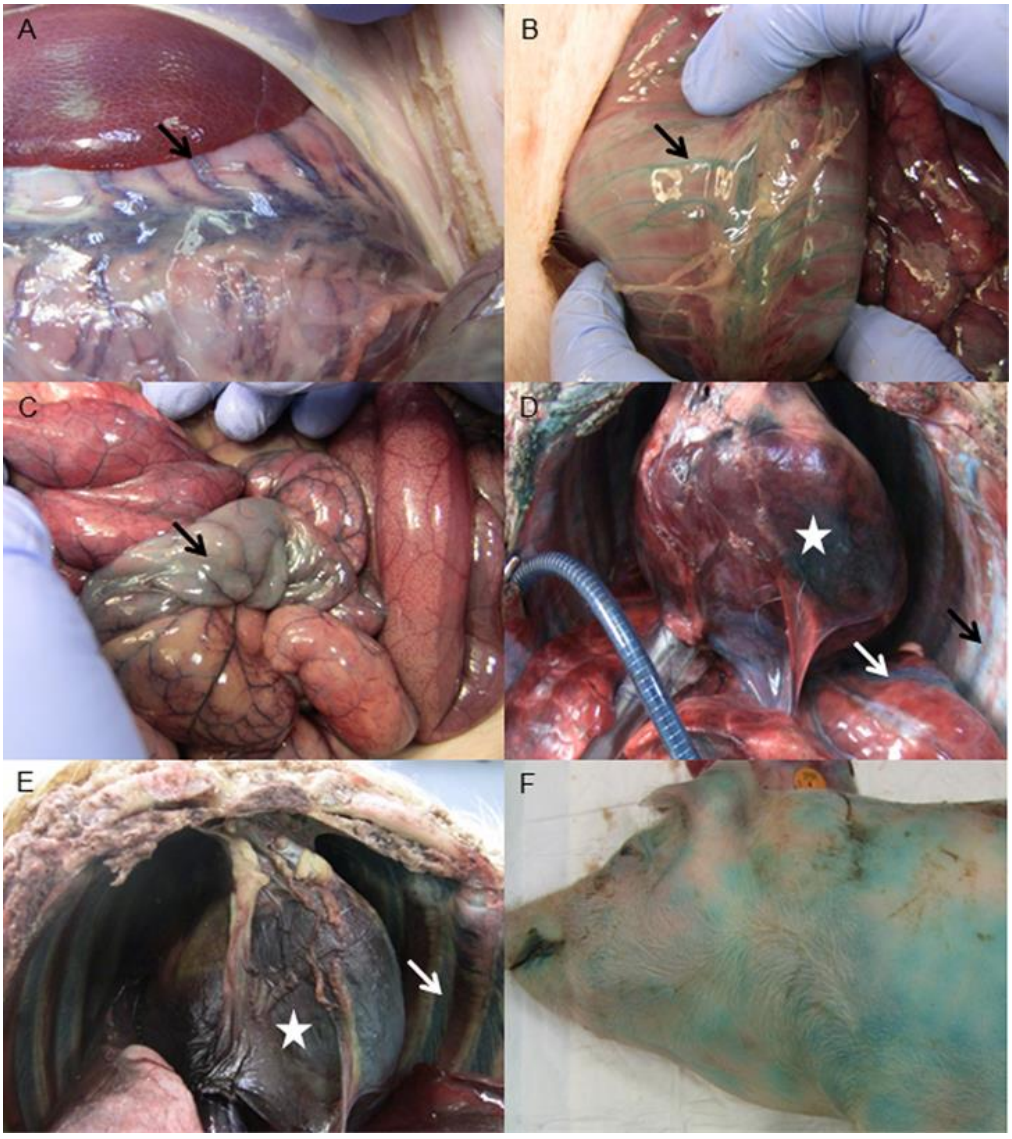

Figure 2. *Spread of Thiel solution in various organs. (A) In the beginning of pump-embalming, branches of the gastroepiploic artery in the gastric greater curvature are filled with light blue embalming solution (arrow), while surrounding veins still contain blood. (B) At the end of the procedure, branches of both gastroepiploic arteries and veins are blue (arrow), demonstrating gastric perfusion. (C) Parts of the small intestinal wall are bluish (arrow), suggesting microvascular spread of embalming solution. (D) During vascular embalming, intercostal vessels (black arrow) and lung vessels (white arrow) turn into bright blue; a blue spot is present at the base of the heart (star). (E) Two weeks after vascular embalming, intercostal muscles (arrow) and heart (star) visibly dyed in blue, indicating ongoing dispersion of embalming product. (F) Two weeks after vascular embalming, blue embalming solution diffuses through the skin.*

In the other cases, PP was distributed at lower flow rates after venous drainage. CT and direct observation revealed that most organs and tissues (*e.g.* skin, stomach, small intestine, colon, mesentery, kidneys and heart) tended to exhibit realistic reperfusion and/or filling of supplying

and draining vessels. The most realistic reperfusion was observed in the kidneys, with preservation of normal gross anatomy and obvious identification on CT of reperfused cortical vessels due to the absence of edema. Arteries and veins in the gastric, intestinal and colonic walls were reperfused without collection formation. Although the gross anatomy of the spleen and liver remained unchanged, these organs were edematous on CT.

PP also leaked into the pleural and abdominal cavities, retroperitoneal space and gastric lumen. In particular, lower halves of both lungs contained excessive PP, resulting in moderate stiffness (Fig. 3). PP did not completely fill the heart cavities. Generally, major vessels (*e.g.* jugular veins, carotid arteries, femoral vessels and coronary arteries) were physiologically swollen, but the caudal vena cava was only partially filled. Moreover, several major abdominal vessels were not visible on CT due to retroperitoneal fluid accumulation. Direct inspection and CT showed arterial and venous filling of the distal vessels in the hind leg without reperfusion of the muscles. Collection formation and organ/tissue appearance during reperfusion with PP are outlined in Table 2 and Figure 4.

Prolonged reperfusion at low flow rates was then established in the specimens (except lower body one) to test several surgical techniques and procedures in realistic circumstances (Fig. 5).

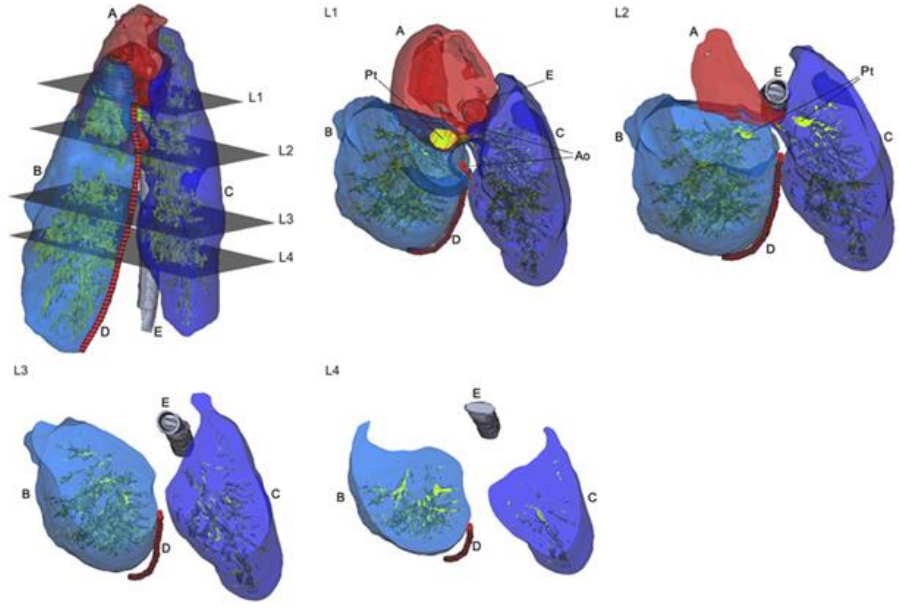

Figure 3. *Reperfusion of Thiel embalmed pig lungs. 3D reconstruction of reperfused lungs in the upper left corner of the figure. Four slices (L1-L4) have been made in cranial-to-caudal direction through the specimen. Top views of the cutting planes L1 to L4 show pulmonary reperfusion from the base to the top of the lungs, resembling the vascular tree structure of the living. Lower halves of both lungs contain more perfusate, causing relative stiffness. A, heart; Ao, aorta; B, left lung; C, right lung; D, arterial tube retrogradely inserted in the descending thoracic aorta; E, draining venous tube placed in the right atrium; Pt, pulmonary trunk and branches.*

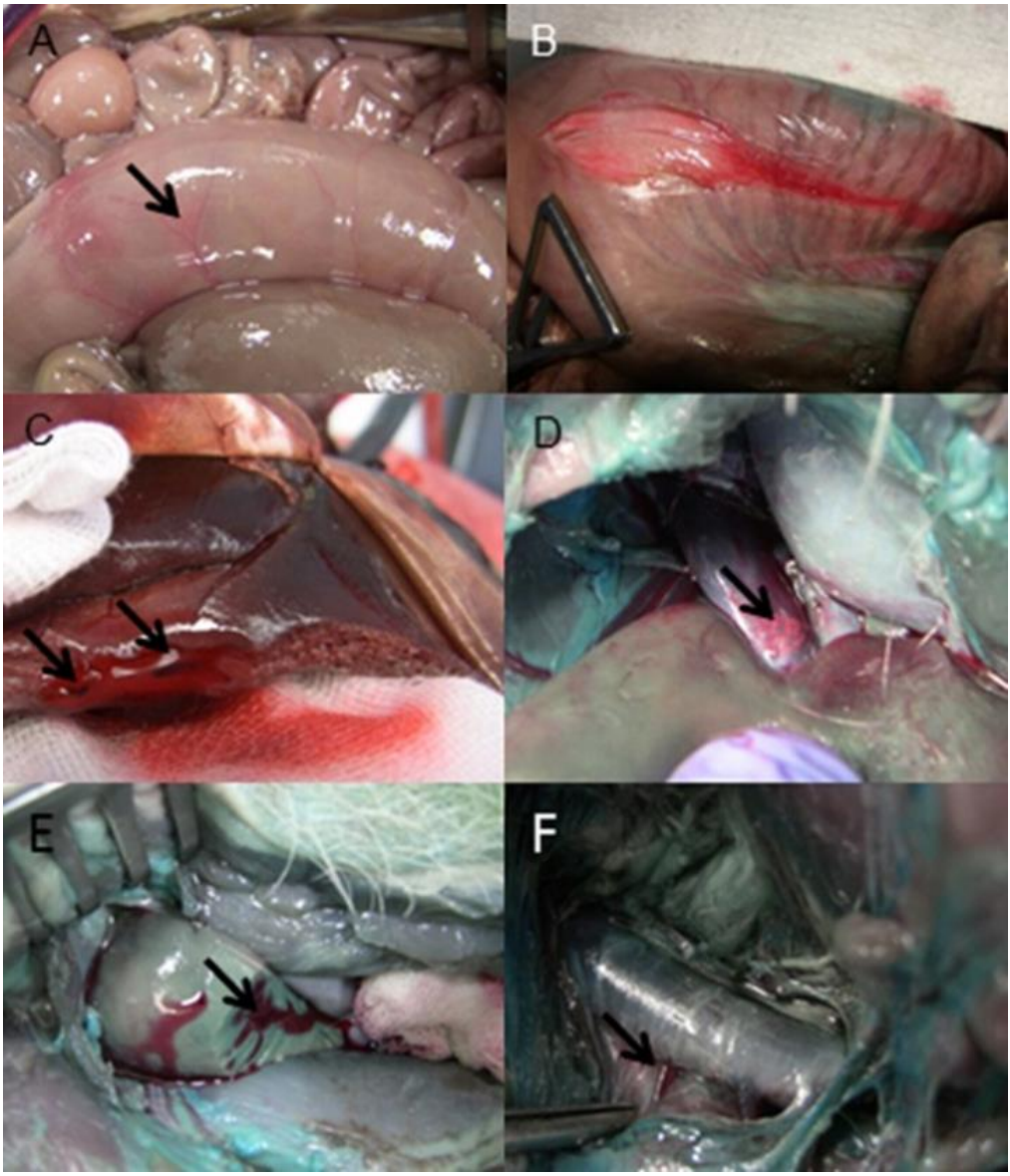

Figure 4. *Reperfusion of dehydrated Thiel embalmed pig models with PP. (A) lifelike appearance of the small intestine. PP recruits the vessels of the intestinal wall (arrow). (B) Simulation of bleeding after incision of the stomach wall. (C) Realistic bleedings (arrows) following hepatic resection. (D) Red PP in the renal vein (arrow) indicates renal reperfusion. (E) Partial nephrectomy. Note the natural bleeding (arrow) and the complete blue staining of the embalmed kidney. (F) External jugular vein and collateral branches filled with PP (arrow).*

Table 2: *Assessment of pig anatomy during vascular reperfusion with PP. PP accumulates in the abdominal cavity, retroperitoneal space and pleural cavity; and leaks into the gastric lumen and airways. The vast majority of reperfused intestinal organs have a normal anatomy, except for liver and spleen, being edematous on CT. PP reperfused major arteries and veins, which have a physiological appearance. *Collections in the small intestinal wall of lower body 1, which can be tackled by diminishing the flow rate. †heart cavities were not completely filled with PP. ‡other major abdominal vessels were not visible because of retroperitoneal fluid accumulation. §PP collected in the gastric lumen. Nl, normal*

Evaluation method	Pleural cavity, peritoneal cavity and retroperitoneal space	Lungs	Stomach	Small intestine	Liver and spleen	Skin, colon and kidney	Carotid, jugular, femoral and renal vessels	Heart and coronaries
Inspection during surgery	Collections	Little stiff	Nl	Nl[1]	Nl	Nl	Nl	Nl
CT	Collections	Edema	Collection[4]	Nl[1]	Edema	Nl	Nl[3]	Nl[2]

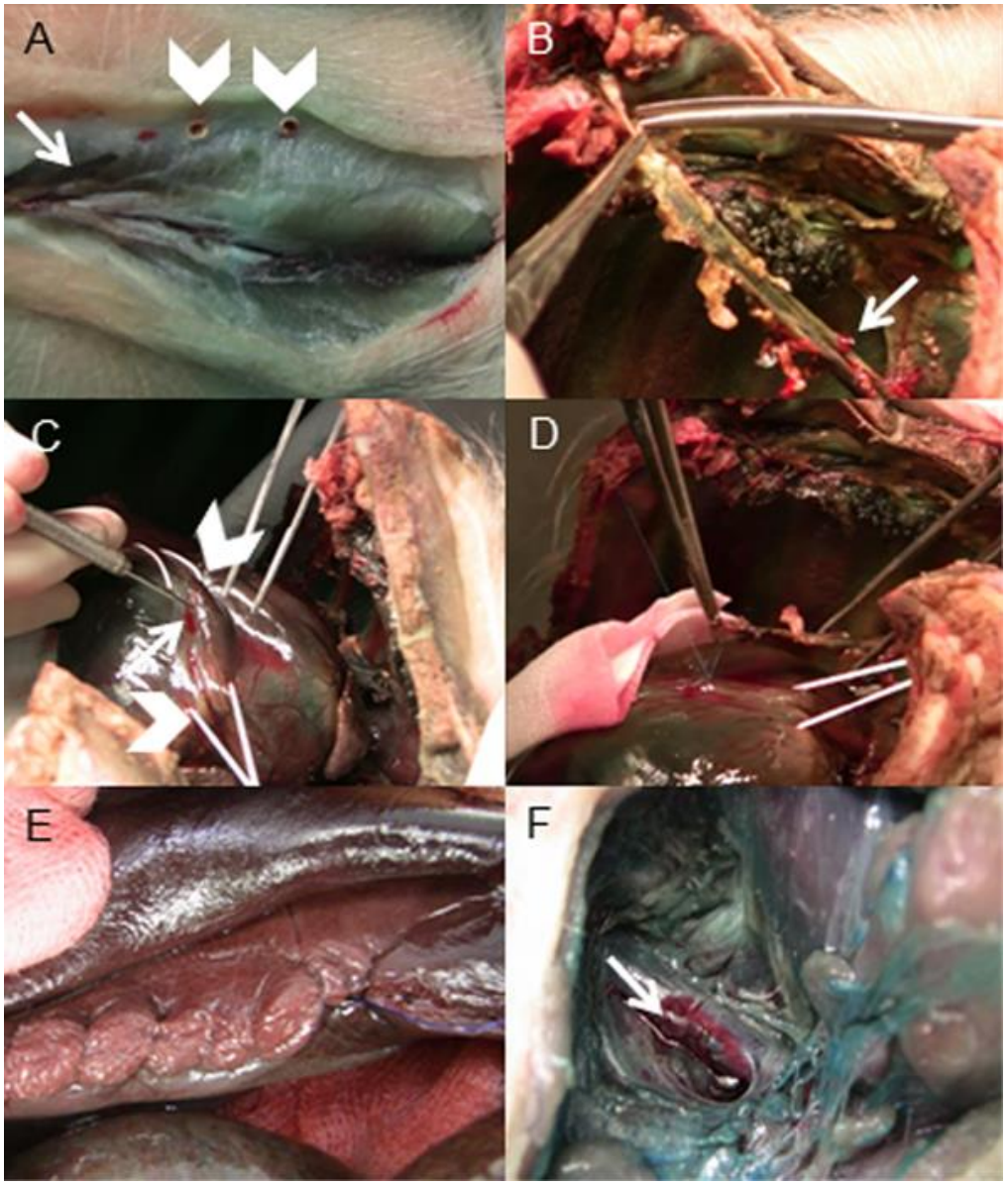

Figure 5. *Surgery performed on a reperfused Thiel embalmed and dehydrated pig model. (A) Bleeding points after incision of the sternal skin can be coagulated (arrowheads) with a coagulation device (arrow). (B) Prelevation of the superior deep epigastric artery to perform a coronary artery bypass grafting. Clips at side branches to control bleeding (arrow). (C) Bleeding after incision of the left coronary artery (arrow). Hemostatic sutures (arrowheads) are needed to do a bloodless anastomosis. (D) Subsequent performance of a coronary artery bypass. (E) Suturing of the inferior liver border after hepatic resection to control hemorrhages. (F) Realistic bleeding after cutting a side branch of the external jugular vein, which must be sutured or clipped (arrow).*

Microvascular reperfusion of pump-embalmed pig small intestines

The embalming solution spread in a fan shape in the arteries of the mesentery and entered the mesenterial fat. It then colored the intestines and ultimately drained through the mesenterial

veins, resulting in adequate embalming of both specimens. The maximum arterial pressure was 30 mmHg.

Macroscopic inspection showed that PP completely filled the vessels of the small intestine and caused realistic coloring of serosa and mucosa. Light microscopy confirmed complete filling of the mesenterial vessels, with gradual spread into the surrounding fat. In particular, PP flowed in the capillaries of the intestinal wall before entering the veins. No extravasation to the interstitial space was observed. Fluorescence microscopy of the intestinal wall demonstrated that PP mixed with DiI recruited a 3D vessel network. Moreover, mesenterial vasculature was clearly visualized by DiI labeling (Fig. 6). The flow of PP was identical in the segment of small intestine prelevated from the third Thiel-embalmed pig.

Microvascular reperfusion of gravity-embalmed human small intestines

Arterially injected PP did not enter the veins. Fluorescence imaging showed that PP was blocked in the arterial branches of the intestinal wall. Light microscopy revealed multiple brownish spots on the mucosa, which probably caused the obstruction. Figure 7 displays these findings and demonstrates the difference between human and pig intestinal mucosa.

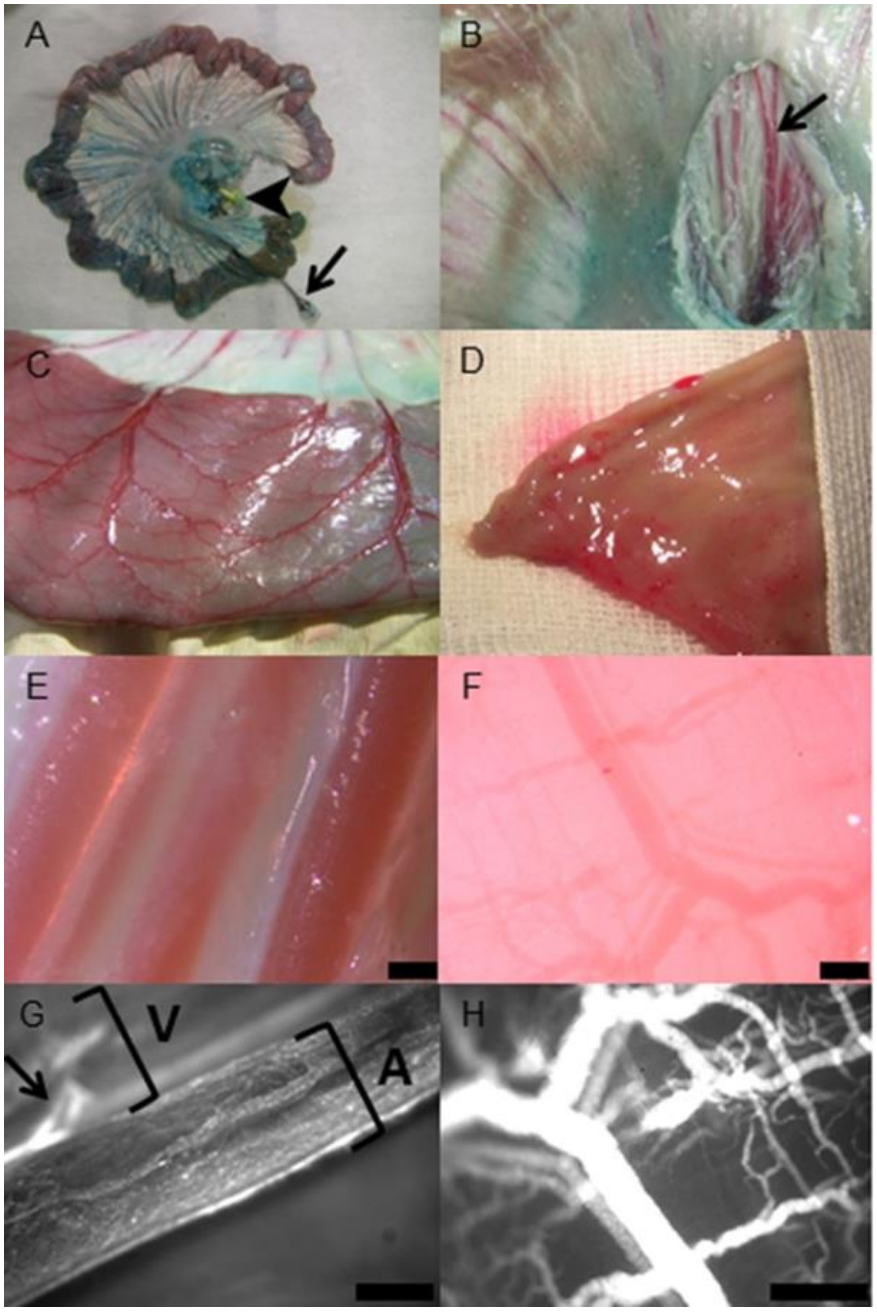

Figure 6. *Propagation of PP in Thiel embalmed pig small intestine. (A) Arterial catheters for perfusate inflow (arrow) and for pressure measurement (arrowhead). (B) Red PP in mesenterial vessels (arrow). (C) PP recruits intestinal wall vessels. (D) Reperfused intestinal mucosa has a natural appearance. (E) Mesenterial and (F) intestinal wall vessels are uniformly reperfused by PP (scale bar 200 µm). (G) DiI binds to the endothelial cells of the mesenterial artery (A), vein (V) and venous valves (arrow) (scale bar; 200 µm). (H) PP and DiI reperfuse the whole vascular network of the intestinal wall (scale bar; 100 µm).*

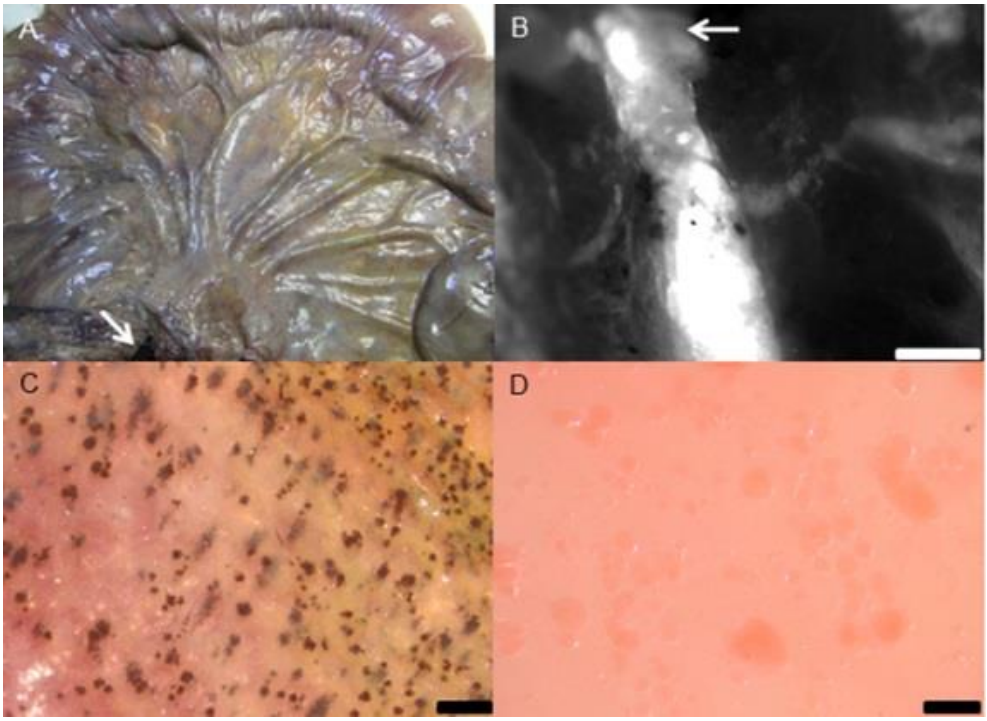

Figure 7. *Difference between Thiel embalmed human and pig small intestines in terms of microvascular reperfusion (scale bars; 200 µm). (A) Thiel embalmed human small intestine. Arterial tube (arrow) to inject PP. Note the expanded vessels. (B) Mixture of PP and Dil does not progress in human intestinal wall vessels (arrow). (C) Light microscopy of human mucosa shows brown spots, suggesting postmortem precipitation of clots and debris. These are fixed in the vessels due to the embalming procedure, hindering reperfusion. (D) Pump-embalmed pig intestines have no mucosal spots.*

Discussion

In this study, we assessed flow parameters during pump-driven Thiel embalming in a pig model. We demonstrated clearly that pressure-controlled pump embalming is rapid because the flow rate can be increased steadily until 65 mmHg, enabling rapid injection of embalming solution. Gravity embalming of human cadavers in our department takes several hours and may be associated with lower intra-arterial pressure and tissue penetration (unpublished observation). We presume that continuous high pressure is essential to enhance tissue perfusion. As the mean arterial blood pressure in adult pigs is 90 mmHg (unpublished observation). Higher pressures may damage vessels, causing more accumulation of embalming solution in the interstitial space. Pump embalming in Thiel cadavers has been reported, but the relevance of pressure measurement has not been emphasized.[37]

The addition of MB permits direct inspection of the spread of embalming solution. Thiel embalming solution has a low viscosity and passes easily through capillary walls.[202] Interestingly, the embalming solution also enters the venous system. The embalming process certainly continues, as we observed progressive blue coloration for a 2-week period. Blue

coloring varied among organs due to different vascularization patterns, but was not associated with inadequate embalming. The natural colors of organs (*e.g.* the spleen is dark) may interfere with this observation. Unexpectedly, the pancreas and greater omentum were found to be brownish, suggesting putrefaction. For this reason, we could not determine whether the embalming solution entered these tissues. Despite generally good tissue preservation, fungal skin infection developed on some distal legs because of incomplete immersion in the Thiel bath. Human cadavers are immersed in a bath to embalm the skin and subcutaneous tissue.[32]

We propose that the presence of embalming fluid in the tissues exerts pressure on the vessels, interfering with subsequent vascular reperfusion. Two osmotic dehydration procedures, – immersion in a Thiel bath and in a concentrated salt solution – partially nullify embalming-induced weight gain and facilitate reperfusion due to high osmolarity (5600 mosmol/l and 10266.7 mosmol/l, respectively; unpublished observation). Immersion in a Thiel bath causes the movement of blood out of the body.[37] In a previous study, the initial weight of organs was restored successfully by dehydration of Thiel-embalmed pig kidneys with a concentrated salt solution **(chapter 8)**.[202]

In this study, we evaluated whether embalming and dehydration enabled reperfusion from artery to vein. We confirmed that arterially injected PP drained through the venous cannula in all specimens. Leakage occurred despite low arterial pressure, but its vascular level(s) remained unclear. Therefore, after initial venous drainage, further perfusate loss can be limited by decreasing the flow rate. As a consequence, vascular reperfusion is possible for hours with limited tissue deformation, enabling surgical training.

Various degrees of leakage cause different anatomical deformations. The kidney had the most realistic reperfusion, with conservation of its gross anatomy. This finding is consistent with the findings of a previous study, in which no increase in weight, volume, or arterial pressure was detected in isolated Thiel-embalmed pig kidneys that were reperfused with PP for more than 1 hour **(chapter 8)**.[202] The reason for this successful reperfusion remains unknown. No fluid accumulated in the gastric, intestinal (except in the lower body of the first pig), or colonic wall, but abdominal collections impeded CT image interpretation. Not unexpectedly, PP leaked into the gastric lumen due to early postmortem autolysis.[87, 204] A retrospective review of 49 multi-phase CT angiographies performed in fresh human cadavers showed extravasation in the gastric lumen in 55 % of cases.[205] In those cases, reperfusion was achieved with PL, which has a viscosity of 137.3 mPa.s at 25°C (personal observation). Of note, the viscosity of PP is 24.9 mPa.s at 25°C (personal observation). We observed no intraluminal discharge in the small intestine or colon, consistent with recent findings.[205] The cause of the significant retroperitoneal fluid content remains unclear, but we assume that this fluid breaks through to the peritoneal cavity, as CT revealed that collections in both spaces were connected. The absence of pump suction at the venous cannula may facilitate accumulation of PP in the inferior pulmonary lobes, while venous drainage of the other lobes is aided by gravity. The origin of leakage into the pleural cavity also remains unclear, as the visceral pleura appeared to be intact. Importantly,

PP reperfused the legs without extravasation, consistent with previous research.[88] Grabherr and co-workers reperfused the lower extremities of two fresh human cadavers with PP diluted with 20 % decane. Venous drainage occurred in the absence of edema in both cases.

Reperfusion of fresh pig organs with tap water for the simulation of laparoscopic surgery has some merit.[86] However, the low viscosity of water (1.0 mPa.s at 20°C) and high pressure ranging from 150 to 180 mmHg are predisposing factors for third spacing of fluid. As a result, deformation is significant and venous reperfusion is limited. Moreover, storage time of fresh tissues is short. Similarly, the majority of researchers have filled the vessels of embalmed human heads or whole bodies with aqueous solutions.[59, 92-95, 97, 170-173, 175, 176, 179]

Unlike water, hydrophobic perfusates (*e.g.* PP) are more confined to vessels and the capacity to extravasate depends on their viscosity. Grabherr et al. established prolonged reperfusion with diesel oil in fresh cadavers of two dogs and one cat.[87]. A chorioallantoic membrane study proved that diesel oil does not enter the capillaries and is not associated with edema. Importantly, capillaries are particularly vulnerable to postmortem permeability. Recently, Grabherr et al established circulation from artery to vein with PL in 45 fresh human cadavers for forensic diagnostic purposes.[90]. Notably, the vast majority of cadavers had only a few drops of perfusate in the gastric lumen. This limited leakage may be explained by the short reperfusion time (6 min) and the high viscosity of PL. In chapter 11 of this thesis we created a simpler model in eight Thiel-embalmed human cadavers using retrograde injection of PL into the femoral artery and expansion of the aorta, enabling the *in situ* placement of aortic valves. As mentioned above, we prefer PP because it leads to no weight or volume gain during prolonged reperfusion in isolated Thiel-embalmed pig kidneys.[202] In the current study, we showed that this successful reperfusion cannot be established in every organ, possibly due to variation in the interaction between PP and tissue at the microscopic level. Pronounced local postmortem autolysis causing capillary wall injury, variable capillary permeability and/or excessive hydrostatic pressure is likely responsible for leakage. In the presence of leakage, ongoing high flow rates will thus lead to unnecessary perfusate loss and tissue deformation.

We demonstrated that reperfused Thiel-embalmed pigs are feasible for surgical training. Hemostasis can be accomplished on reperfused skin and subcutaneous tissue, like in patients or live animal experiments. This skill can also be practiced on reperfused human training models using tap water.[92, 95, 170] Dissection of physiologically filled vessels and performance and evaluation of vascular anastomoses can also be performed. Other authors have reported on vessel dissection and arterial anastomosis[94, 170, 172, 175], but venous anastomosis is feasible only if veins are filled, for example, after creating arteriovenous shunts.[95] Importantly, as PP spreads in solid organs, surgical resection requires adequate control of feeding arteries and draining veins. No previous report has described *in situ* dissection and removal of reperfused embalmed organs. Moreover, parenchymatous bleeding and major bleeding following large vessel laceration during partial kidney or liver resection occur and hemostasis can be judged. Similar simulations can be accomplished briefly during reperfusion of isolated fresh pig organs.[86] Of

note, Aboud et al. created arteriovenous shunts in a novel human cadaver model for trauma surgeon training and simulated comparable life-threatening organ bleeding.[95] However, the cadavers were stiff, impeding organ resection. Our model is also suitable for stapling device development because bleeding after intestinal resection can be simulated and hemostasis can be tested.

The investigation of microvascular reperfusion in pump-embalmed pig small intestines and comparison with that perceived in gravity-embalmed human tissues, are essential to improve this model. In small intestines that were pump embalmed before and after surgical resection, PP ran in the total vascular network of the pig mesentery and intestinal wall and did not extravasate. In both models, agony and embalming circumstances may be ideal to facilitate vascular reperfusion; pressure-controlled pump embalming performed shortly after death in young animals with patent vessels may minimize autolysis and capillary damage and provides vessels free of clots and postmortem debris. In addition, PP generates low arterial pressure in the intestinal model, minimizing vessel wall damage. In contrast with this observation, multiple brownish spots observed in the mucosa of gravity-embalmed human small intestine may obstruct arteries. These spots likely represent postmortem clots and cellular debris fixed by formalin. The presence of postmortem clots depends on the duration of agony and timing of postmortem embalming.

Capillary reperfusion can be considered to be a disadvantage because leakage from the capillaries probably occurs. Substantial flow with viscous PL may be associated with less capillary reperfusion, but also generates higher arterial pressure, which may cause vessel wall damage. Furthermore, when capillaries are not entered, flow from artery to vein depends on the presence of arteriovenous shunts in the organs and tissues.

This study has several limitations. Computed tomography was performed after 80 min reperfusion, allowing accumulation of PP. This accumulation impeded interpretation and visualization of tissue reperfusion. The absence of arterial pulsation is a shortcoming, but it can be established with a modified intra-aortic balloon pump.[59, 95-97, 170, 172, 176] In addition, CT-based 3D reconstruction of the embalmed organ(s), as illustrated in Figure 3, showed clear underestimation of the reperfusion process. The gray value distribution pattern indicates diffuse capillary-like filling. However, attempts to segment this diffuse filling resulted in a solid, opaque 3D reconstruction, with no relevant insight. We thus chose a segmentation strategy using a grayscale threshold corresponding to an approximate 0.8-mm vessel diameter cutoff, resulting in visualization of an illustrative multibranching structure.

In conclusion, the current study provides useful insight for the establishment of prolonged reperfusion in Thiel-embalmed tissues with limited anatomical damage, allowing realistic anatomy instruction and surgical training. We recommend the performance of pressure-controlled pump embalming as soon as possible in the postmortem phase. The exceptional perfusion properties of PP depend on the quality of embalmed tissue, vascular patency, type of capillary, presence of arteriovenous shunts and established vascular pressure.

PART F

Preliminary results of long dynamic Thiel embalmed human tissue reperfusion

Chapter 10

Preliminary results of dynamic reperfusion of Thiel embalmed humantissues

Introduction

The knowledge and experience gained during the previous experiments on pigs was applied to establish reperfusions in Thiel embalmed human tissues. In this chapter, preliminary results are shown in a series of ten kidneys, one liver, one arm and one total body. This research focuses whether it is possible to install a prolonged reperfusion from artery to vein in these specimens. Vascular flow is assessed in terms of flow rate, vascular pressure and venous drainage. Tissue effects are evaluated through external gross anatomical deformations; weight and volume changes; and CT graphic findings. Lastly, feasibility for surgical training is observed in the reperfused arm.

Kidneys

Materials and Methods

Ten kidneys were prelevated from five gravity-embalmed Thiel cadavers. Kidneys were chosen because these organs have proven to be a suitable model to study vascular reperfusion **(chapter 8)**. Particular attention was paid to preserve sufficient length at the level of the renal artery and vein, allowing vascular puncture and cannulation. Moreover, perinephric fat was not resected (in 8 out of 10 cases), insuring as little as possible renal damage. The kidneys were weighed and their volumes were measured by immersion in a vessel full of water (Archimedes' principle). Initially, a Quik-Cath II 14-gauge catheter (Baxter, Mayo, Ireland) was inserted in the renal artery and attached to a tube, which was placed in a roller pump (Watson-Marlow 520 U, Zwijnaarde, Belgium). The renal vein was also cannulated and the ureter was ligated.

A reservoir was filled with 100 cc red PP. The pump then injected PP into the kidney at a flow rate of 1 mL/min. Immediately after initiating the reperfusion, the renal artery was punctured with a BD Insyte-W 22-gauge catheter (BD Vialon, Madrid, Spain) and connected to an ultraminiature fibre optic pressure transducer (Samba 201 CAP, Harvard Apparatus, Cedex, France), permitting a maximum arterial pressure of 50 to 60 mmHg. Time until venous drainage was noted. At the time of venous outflow, the catheters were disconnected and the renal volume and weight were determined. After reconnecting the catheters, closed pressure-controlled circulation with PP was re-established, *i.e.* the venous outflow in the reservoir was pumped into the renal artery anew. Pressures were noted at the beginning of the procedure and then every quarter until termination of the experiment. The reperfusion was interrupted after 60, 120, 180 and 240 minutes to measure the renal volumes and weights. Afterwards, the renal appearance (swelling, pliability, capsular leakage and subcapsular collections) was assessed. Reperfusion restarted after adding contrast agent Angiofil® to the remaining PP in the reservoir (amount added was 10 % of the volume of PP). CT was performed to analyze the vascular distribution of PP.

Subsequently, a more lifelike reperfusion in every kidney was intended. Therefore, after reconnecting the catheters and cannulas, the flow rate was gradually increased over five minutes to 25 mL/min. This flow rate persisted for 60 minutes, during which arterial pressures were monitored and noted every three minutes. Moreover, venous drainage (mL/min) was measured after 30 and 60 minutes. As described above, renal appearance and the vascular dissemination of PP were investigated again.

Finally, the effects of reperfusion on tissue morphology were evaluated. Therefore, cortical biopsies were taken from Thiel embalmed kidneys; after 240 minutes of reperfusion at 1mL/min; and after 60 minutes of reperfusion at 25 mL/min. The biopsies were stained with orcein as well as hematoxylin and eosin.

Statistical Analysis

Statistical analysis was done with SigmaPlot Version 13. Comparisons of the results of the measurements at different times were performed with the Friedman and Holm-Sidak tests. A P value < 0.05 was deemed statistically significant.

Results

Mean time until venous drainage was 13.5 min (range: 4-23). There was a statistically significant weight gain noted between 0 and 2 hours of reperfusion ($P < 0.010$). No statistically significant weight difference was encountered between 2 and 4 hours of reperfusion ($P = 0.937$). No statistically significant volume and pressures changes were observed between initiation and termination of the reperfusion ($P = 0.056$; $P = 0.393$, respectively). The results are illustrated in figure 1. Every reperfused kidney remained pliable. Parenchymatous leakages were found in three cases at the level of biopsy. Collections were found at the hilus ($n = 1$) and under the renal capsule ($n = 6$). CT imaging demonstrated good visualization of major renal arteries up to the interlobar arteries, however, arcuate arteries were only moderately depicted (fig. 2). Smaller vessels were not visualized. Limited perfusate accumulations were observed in the cortex. Histological analysis and CT reconstructions (also to assess venous reperfusion) are awaited for the end of 2015.

Subsequent flow at a rate of 25 mL/min caused statistically significant weight and volume gain ($P < 0.001$; $P = 0{,}004$, respectively), but no statistically significant pressure difference ($P = 0.69$) was measured (fig. 3). Venous flow rates after 30 and 60 minutes were 13.72 mL/min (range: 1.5-20) and 15.06 mL/min (range: 7-21), indicating leakage that was present in every specimen either at the parenchymal surface or at the hilus. Most kidneys remained supple except for two unknown statuses and one specimen that became a little stiff. At least in two cases, PP entered the renal pyelum.

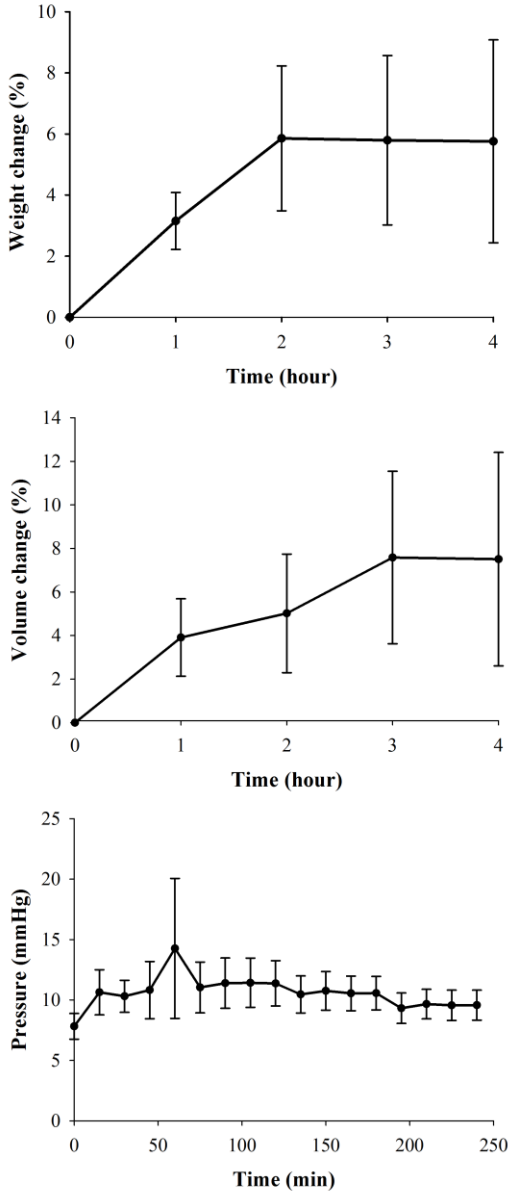

Figure 1. *Prolonged reperfusion at low flow rate. (upper graph) Weight change, (middle graph) volume difference and (lower graph) arterial pressure during four hours of reperfusion at 1 mL/min.* There is a significant weight gain between 0 and 2 h ($P < 0.010$). No statistically significant weight difference was encountered between 2 and 4 h ($P = 0.937$). No statistically significant volume and pressures changes were observed between initiation and termination of the reperfusion ($P = 0.056$; $P = 0.393$, respectively).

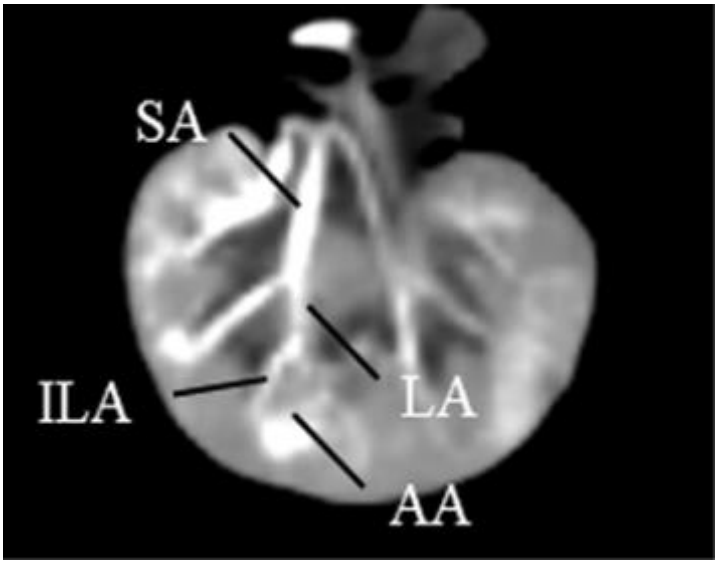

Figure 2. *Vascular distribution of contrast-enhanced PP in a Thiel embalmed human kidney. Major renal vascular anatomy has been nicely preserved, but arcuate vessels were moderately imaged. SA, segmental artery; LA, lobar artery; ILA, interlobar artery; AA, arcuate artery.*

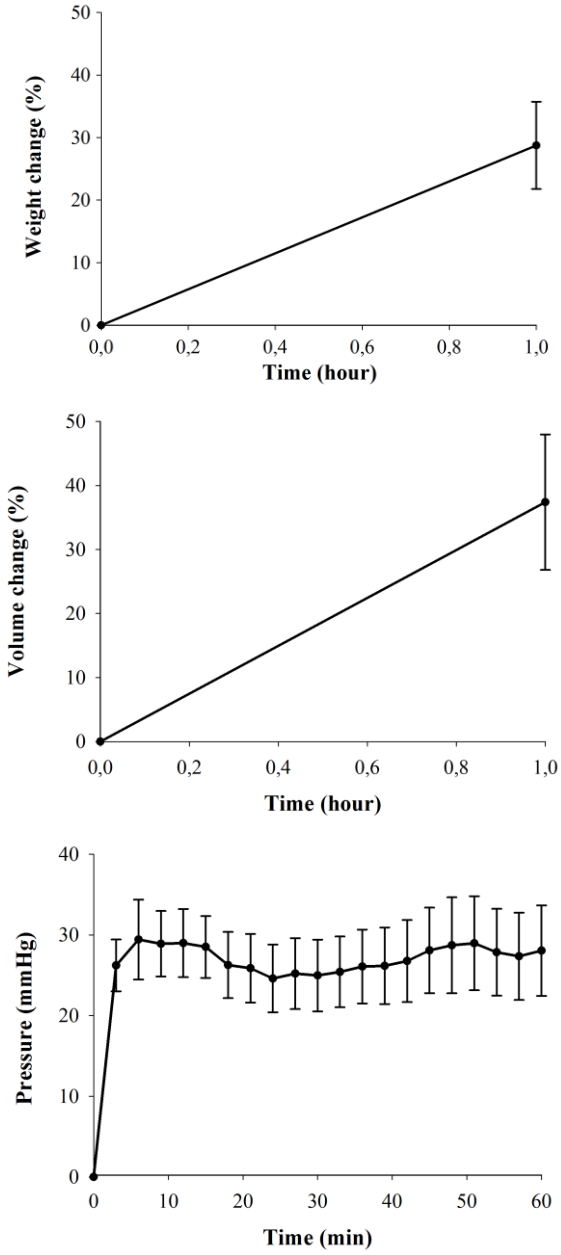

Figure 3. *Reperfusion at high flow rate. (A) Weight change, (B) volume difference and (C) arterial pressure during one hour of reperfusion at 25 mL/min.* Statistically significant weight and volume gain ($P < 0.001$; $P = 0{,}004$, respectively), but no statistically significant pressure difference ($P = 0.69$) were measured.

Liver

Materials and methods

One liver, weighing 1,867 gram was removed from a gravity-embalmed human cadaver. The hepatic artery and portal vein were identified and cannulated with a Quik-Cath II 14-gauge catheter. Both catheters were connected to a three way stopcock, which was attached to a tube that was placed in a roller pump. The portal vein was then punctured with a 22-gauge catheter and connected with the pressure transducer, permitting a maximum pressure of 50 to 60 mmHg. The inferior part of the caudal vena cava was also cannulated with a large tube, while its superior part was closed with a running suture, insuring unidirectional venous drainage. Eventually, the choledochal duct was searched for and ligated.

Subsequently, the reservoir of the pump was filled with 300 cc red PP and a hepatic reperfusion was established at a flow rate of 10 mL/min until venous drainage. Afterwards, during continuous pressure monitoring, flow rate was gradually increased. At the end of the experiment, a mixture of red PP and 10 % Angiofil® was pumped into the liver and the vascular distribution of PP was CT graphically visualized. The general status of the liver (leakage, collections and suppleness) was assessed at the end of the procedure.

Results

PP flowed at a rate of 25 mL/min for 240 minutes. The maximum vascular pressure was 30 mmHg. Although drainage in the venous cannula was established, gallbladder swelling and some leakages at damaged parenchyma occurred. The liver, however, preserved its natural color, retained its suppleness and did not have external collections or deformations (fig. 4A). CT imaging showed an irregular network of reperfused vessels and local perfusate accumulation without clear anatomical landmarks (fig. 4B).

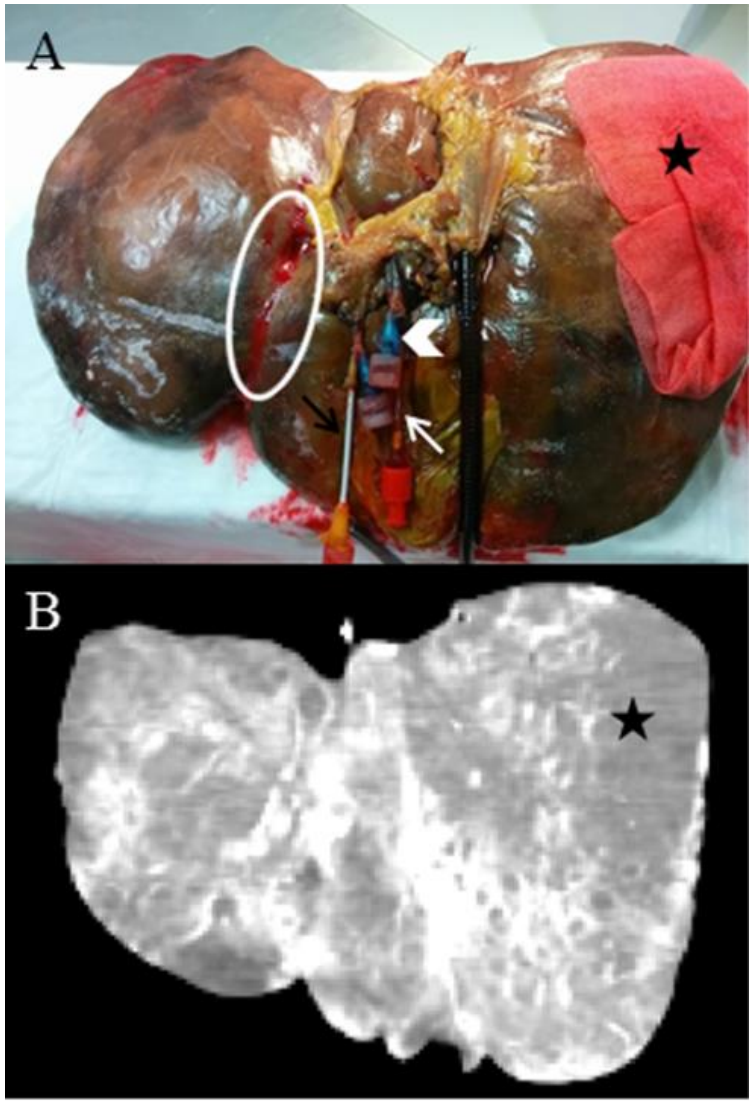

Figure 4. *Reperfused Thiel embalmed human liver. (A) Liver status after 4 hours of reperfusion with PP. Catheters are inserted in the hepatic artery (black arrow) and portal vein (white arrow). The portal vein is punctured with a pressure probe (arrowhead). Small (circle) and major (star) lifelike parenchymatous bleedings are present. (B) CT of the same liver. PP locally accumulates. The vascular anatomy of the liver is unrecognizable. The region without contrast (star) corresponds with major parenchymatous damage (star in picture A).*

Arm

Materials and methods

One human arm of 2,750 kg removed from a gravity-embalmed Thiel cadaver was cannulated at the level of the axillary artery and vein. Initially, a reservoir was filled with 400 cc red PP. As previously described, a roller pump injected PP for 240 minutes into the arterial access under continuous pressure monitoring. The flow was gradually increased until 10 mL/min without exceeding arterial pressures of 50 to 60 mmHg. During reperfusion, skin staining, superficial vein swelling and source of perfusate leakage were noted. After 240 minutes, a mixture of 6 % Angiofil® and 94 % PP was pumped into the specimen. The flow of PP was then visualized with CT. Later, vascular surgical procedures (*i.e.* vessel dissection, arteriotomy closure and anastomoses) were practiced on this reperfused model.

Results

Arterially injected PP easily drained via the cannula in the axillar vein. A flow rate of 10 cc was established during 240 minutes, during which a maximum arterial pressure of 42 mmHg was noted. Significant leakage was only found at the cutting edges of the upper arm muscles. Inspection revealed no particular deformations, apart from local skin staining due to gravity-induced accumulation of PP in the subcutaneous tissues (fig. 5). This observation was confirmed on CT (fig 6). The skin of the fingers remained pale. This is in accordance with CT findings, showing no filling of the proper palmar digital arteries. However, small vessels in the hand were reperfused. CT evaluation and direct inspection of the skin demonstrated reperfusion of both deep and superficial venous systems of the arm. Figure 6 illustrates the vascular distribution of PP.

Skin incision, tissue dissection, hemostasis, vessel manipulation and isolation were performed under realistic conditions. Like in patients, before vessel incision, vascular clamps or hemostatic sutures were placed. Then, lifelike vascular anastomoses and arteriotomy closures were practiced. Finally, quality of vascular suturing could be evaluated. Figure 7 stepwisely depicts dissection of the radial artery, arterial incision, suturing of the arteriotomy and assessment of the closure.

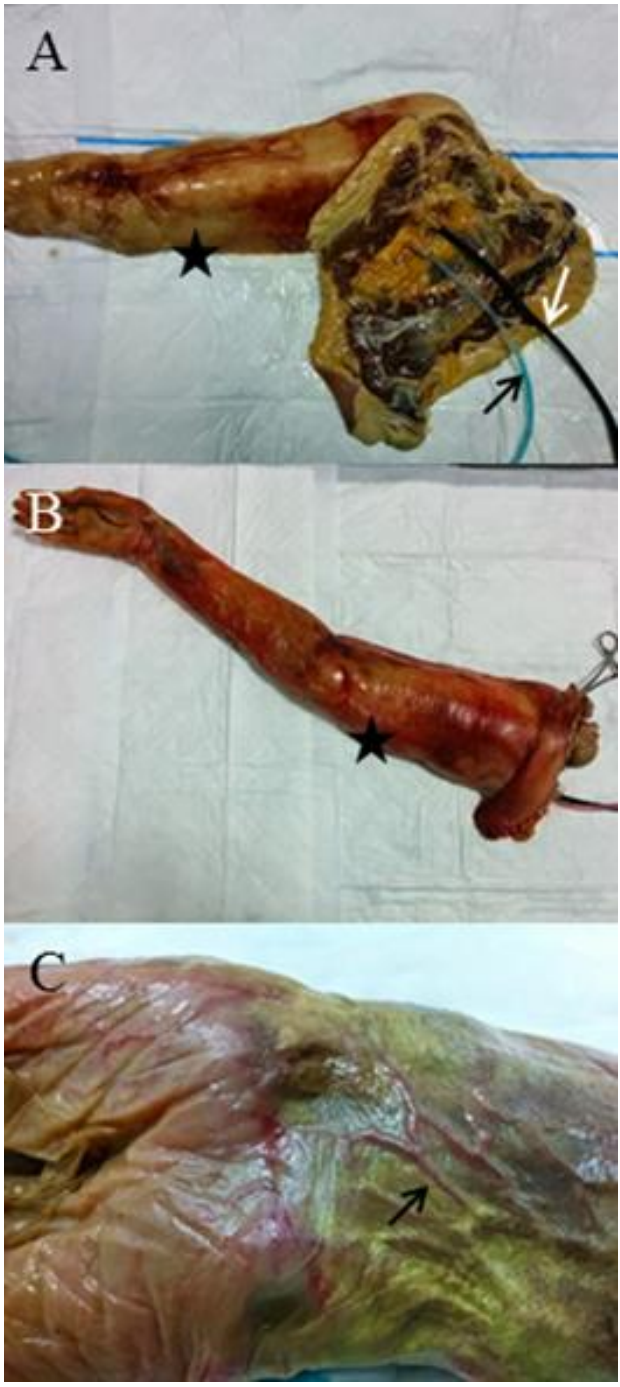

Figure 5. *Reperfusion of a Thiel embalmed human arm. (A) Thiel embalmed human arm before reperfusion. Cannulas are placed in the axillary artery (white arrow) and axillary vein (black arrow). Skin of the upper arm is locally pale (star). (B) Reperfused Thiel embalmed arm. Skin turned red (star). (C) Reperfused veins at the ventral part of the wrist.*

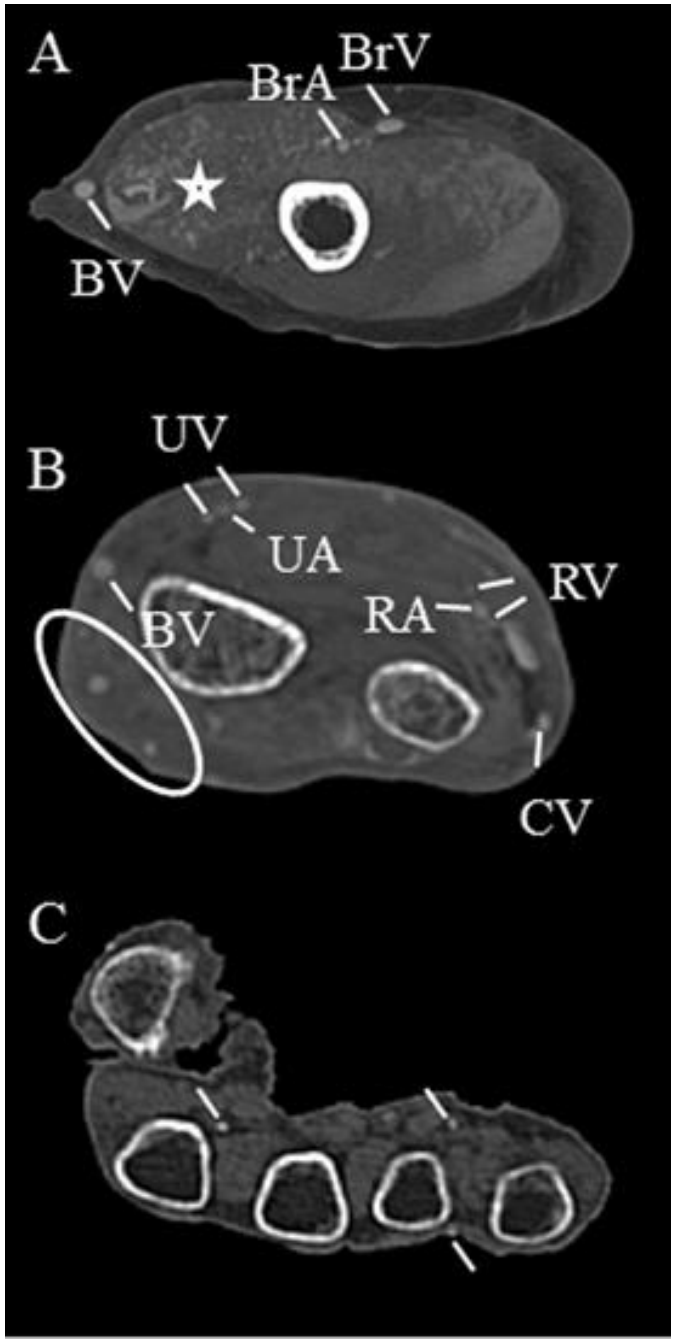

Figure 6. *CT of a reperfused Thiel embalmed arm. (A) Cross section of the upper arm. Arterial and venous (superficial and deep) systems are filled with contrast-containing PP. PP entered the muscle vessels (star). (B) Cross section of the lower arm. All major vessels, including dorsal veins (circle), are reperfused. (C) Cross section of the hand. Minor vessels are reperfused (line). BrA, brachial artery; BrV, brachial vein; BV, basilica vein; CV, cephalic vein; RA, radial artery; RV, radial vein, UA, ulnar artery; UV, ulnar vein.*

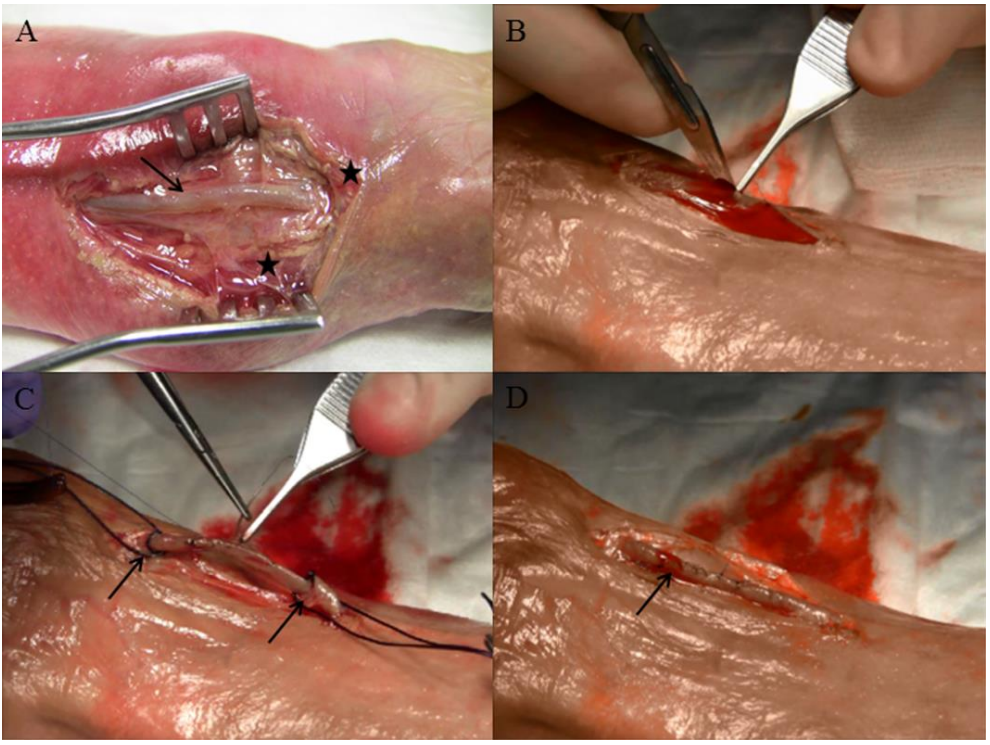

Figure 7. *Dissection, incision, suturing and evaluation of a sutured reperfused radial artery. (A) Dissection of a reperfused radial artery. The radial artery has a physiologic appearance (arrow). Hemorrhages (stars) occur during dissection. (B) Incision of the radial artery causes lifelike major bleeding. (C) Suturing of the arteriotomy. Hemostatic sutures (arrows) are required to perform a bloodless suturing. (D) Evaluation of the suturing quality. A small bleeding (arrow) persists, necessitating an extra suture.*

Total body

Materials and methods

One fresh human cadaver was embalmed with the pump 44 hours after death. Therefore, 15,958 cc Thiel embalming solution was retrogradely flowing in the femoral artery at a rate of 172 mL/min using a 14-gauge catheter. This was followed by embalming in a Thiel bath for 6 weeks and CT graphic imaging of the cadaver.

Subsequently, a draining tube was inserted in the femoral vein in antegrade direction. The cannula in the femoral artery was then connected to a tube that was placed in a roller pump. Simultaneously, trocars were inserted in the abdomen to install a pneumoperitoneum, allowing inspection of the reperfusion. Similarly, trocars were placed in both pleural cavities. The pump then injected 1,900 cc red PP mixed with 6 % Angiofil® retrogradely in the femoral artery at increasing flow rates. The maximum permitted arterial pressure was 50 mmHg.

Results

The arterial flow rate was gradually increased to 30 mL/min. The reperfusion lasted 93 minutes, during which the arterial pressure ranged from 3.3 to 15.2 mmHg. After 25 minutes, first signs of reperfusion were observed in the abdomen (vessels in the intestinal wall, ligamentum falciforme and omentum majus). After 40 minutes, cutaneous vessels turned red at the face, shoulders, arms and dorsal side of the hands. At the end of the arterial injection, vessels in the diaphragm muscle were reperfused; further swelling of cutaneous veins was detected; a bleeding arterial ankle ulcer was noted; and limited accumulation of perfusate in the peritoneal cavity and mesentery were found. Because of lack of drainage in the venous cannula, 900cc red PP mixed with 6 % Angiofil® was then antegradely injected in this cannula at 30 mL/min. Inspection during venous reperfusion showed coloring of pericardial and epigastric vessels, further filling of diaphragmatic vessels, staining of the lung bases and significant swelling of the retroperitoneal space in the pelvis (fig. 8). Because of retroperitoneal leakage, no dynamic flow (from artery to vein) was attempted. Afterwards, the cannulas were closed to prevent perfusate leakage. Later, a whole body CT was performed to assess the intracorporeal dispersion of PP (fig. 9 and 10).

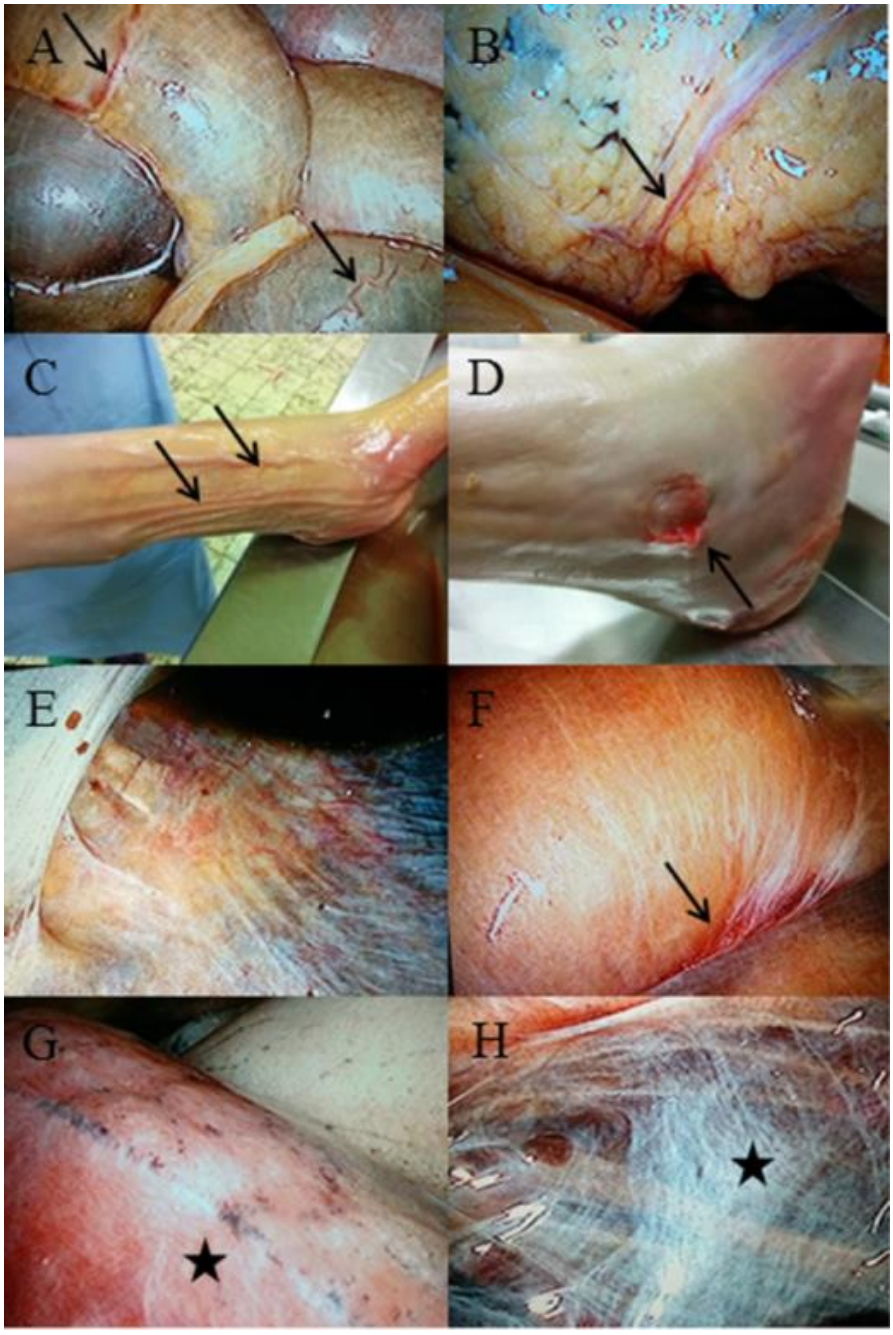

Figure 8. *Consecutive observations during reperfusion of a pump-embalmed Thiel cadaver. Initial arterial reperfusion (arrows) of the intestinal wall (A) and omentum majus (B); At the end of arterial reperfusion: progressive swelling of subcutaneous arm veins (arrows) (C), a bleeding arterial ulcer (arrow) (D), diffuse reperfusion of the diaphragm muscle (E) and accumulation of PP (arrow) in the mesentery (F) were observed; At the end of the venous reperfusion: diffuse staining of both long bases (G) and ongoing perfusate accumulation in the retroperitoneal space of the right fossa (H) occurred.*

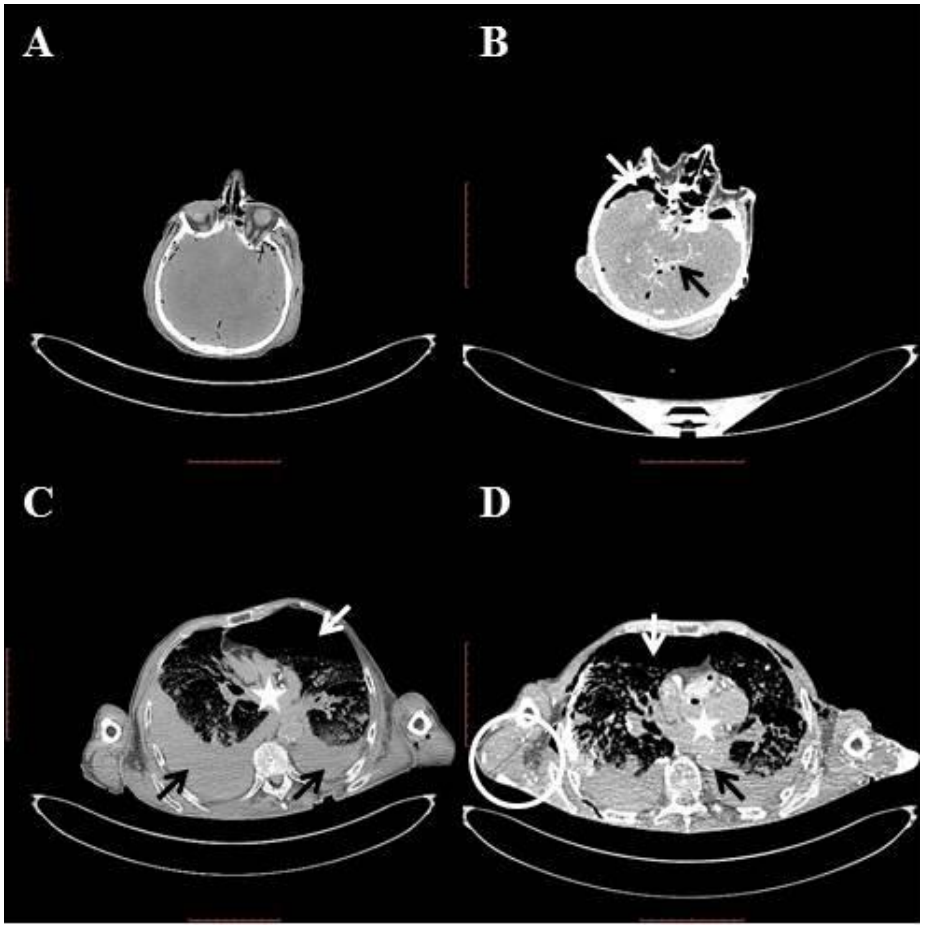

Figure 9. *Anatomical effects of on-pump reperfusion of a Thiel embalmed human cadaver with contrast-enhanced PP. (A) Brains before reperfusion; (B) brains after reperfusion: cerebral vessels (black arrow) filled with PP, free air intracranially meaning that brains have lost volume (white arrow); (C) thorax before reperfusion: embalming fluid accumulates in the pleural cavities (black arrows), left pneumothorax (white arrow) due to blood sampling in the subclavian vein before embalming, heart cavities are shrunken (star); (D) thorax after reperfusion: bilateral pneumothorax after thoracoscopy (white arrow), reperfused vessels in the upper arm and shoulder (circle), aorta contains no contrast because of spontaneous drainage into side branches (black arrow), heart has regained its original volume (white star).*

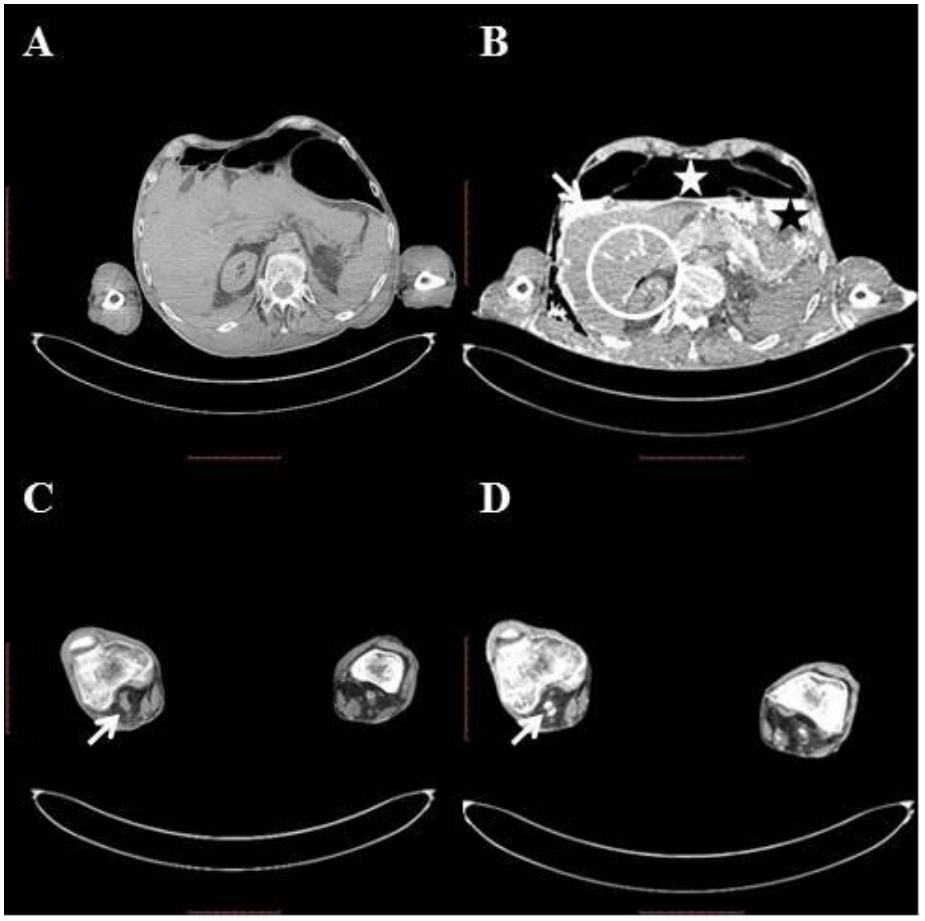

Figure 10. *Anatomical effects of on-pump reperfusion of a Thiel embalmed human cadaver with contrast-enhanced PP. (A) Abdomen before reperfusion; (B) abdomen after reperfusion: PP enters vessels in kidney and liver (circle), PP accumulates in the stomach (black star), free air in the abdomen after laparoscopy (white star), free PP intraabdominally (white arrow), suggesting leakage; (C) legs before reperfusion: popliteal vessels (arrow); (D) legs after reperfusion: popliteal artery and vein contain contrast.*

Discussion

This research investigates whether it is possible to install a prolonged reperfusion from artery to vein in several types of Thiel embalmed human tissues. We demonstrate that this is feasible in one isolated arm, kidneys and one liver. Although venous drainage is clearly visible in the arms, legs and face during reperfusion of a complete body, no outflow occurs through the cannula in the femoral vein. Several causes for this observation can be put forward. Firstly, postmortem vascular permeability varies among human tissues due to vascular obstructions and presence of arteriovenous shunts.[206, 207] We have previously shown **(chapter 9)** that venous drainage could not be established in Thiel embalmed human intestines, probably due to postmortem embalmed debris that gets stucked in the mucosal vessels. Accordingly, laparoscopic

inspection during arterial reperfusion showed mesenterial accumulation of PP that later expanded toward the retroperitoneal space in the pelvis, indicating forward obstruction. Secondly, postmortem capillary fenestration may have caused perfusate loss toward the interstitial space, diminishing venous return. Although this hypothesis has not been demonstrated in the completely reperfused body it is present in the subcutaneous tissues of the arm, renal cortex and liver parenchyma. Thirdly, no venous cannula was inserted in the femoral vein during the embalming procedure. As a consequence, no outflow of excess blood and clots in the major veins occurred, blocking complete venous reperfusion and adequate drainage of PP.

The established vascular flows and associated pressures are clearly lower than in patients. This cautious attitude is essential to limit perfusate loss and tissue deformation, which - as described above - may not be avoided. Note that after the flow rate has been increased by 25 times in the kidney, the pressure rises by approximately factor 3, demonstrating the large arterial adaptation capacity. However, this is associated with significant weight and volume gain. Although, no CT imaging of the kidney was performed after reperfusion at high flow rate, perhaps growing cortical collections are responsible for this phenomenon.

No weight and volume increase occur during long renal reperfusion at low flow rates. Moreover, no major anatomical deformations are present in the other reperfused organs. Despite these good results, CT imaging shows local (kidney and arm) and diffuse (liver) collections. As we intend to use these reperfused models for surgical training, this CT graphic finding is not a major issue.

Feasibility for surgical training is assessed in a softly preserved arm model. We demonstrate that open vascular surgery can be practiced in realistic circumstances. Further analysis as a surgical training tool is, however, necessary.

Conclusion

PP runs for hours from artery to vein in isolated human kidneys, one liver and one arm, whereby avoiding gross anatomical deformations due to low established vascular pressures. Effective venous drainage is noted locally in a complete human body, indicating that vascular reperfusion is a complex process that differs among tissues. Successful venous drainage in a complete Thiel body depends on absence or incomplete vascular obstruction, limited capillary wall permeability, low vascular pressure and probably arteriovenous shunts. In addition, pump-embalming using a venous cannula may aid to achieve effective venous drainage of blood. Reperfused Thiel embalmed tissue seems to be an interesting surgical training tool.

PART G

Arterially pressurized Thiel embalmed cadavers: an essential model for training

Chapter 11

Postmortem circulation: a new model for testing endovascular devices and training clinicians in their use

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Abstract

The development of new medical devices, such as aortic valves, requires numerous preliminary studies on animals and training of personnel on cadavers before the devices can be used in patients. Postmortem circulation, a technique used for postmortem angiography, allows the vascular system to be reperfused in a way similar to that in living persons. This technique is used for postmortem investigations to visualize the human vascular system and to make vascular diagnoses. Specific material for reperfusing a human body was developed recently. Our aim was to investigate whether postmortem circulation that imitates *in vivo* conditions allows for the testing of medical materials on cadavers. We did this by delivering an aortic valve through the femoral artery (*i.e.* transcatheter aortic valve implantation (TAVI)). Postmortem circulation was established in eight corpses to recreate an environment as close as possible to *in vivo* conditions. Mobile fluoroscopy and a percutaneous catheterization technique were used to deliver the material to the correct place. Once the valve was implanted, the heart and primary vessels were extracted to confirm its position. Postmortem circulation proved to be essential in several of the cadavers because it helped the clinicians to deliver the material and improve their implantation techniques. Due to the vascular circulation, sites with substantial arteriosclerotic stenosis could be bypassed, which would have been impossible without perfusion. Although originally developed for postmortem investigations, this reperfusion technique could be useful for testing new medical devices intended for living patients.

Introduction

The development of transcatheter technology and, more specifically, transcatheter aortic valves requires the preclinical use of animal models to study the performance of the implant in acute and chronic situations. Unfortunately, there are fundamental anatomical differences between animal models and humans. None of the current animal models allow for adequate evaluation of the position, deployment, anchoring and functioning of transcatheter aortic heart valves in the orthotopic position. The main limitations of these models are the short distance between the ostia of the coronary arteries and the aortic valve annulus, the small distance between the aortic valve annulus and the mitral valve leaflets (absence of an aortic–mitral hinge), the relatively short length of the ascending aorta and the angle of the aortic arch curvature.[208] In addition to these anatomical differences, the absence of calcification, which characterizes severe calcific aortic stenosis, makes the model inappropriate for evaluating and assessing the fixation and migration (anchoring) behavior of the implant.

To overcome these anatomical and etiological limitations, a more appropriate model is needed. Human cadavers have been used to study the placement of endovascular devices and to assess how they might interact with the local anatomy. The use of cadavers for this purpose has been limited because it was only possible by means of surgical access, which limits the relevance of the findings to the performance of devices normally placed by endovascular delivery. Therefore, the advent of a new technology that allows endovascular procedures to be performed on human cadavers would be of great benefit to researchers.

In 2008, Grabherr et al. introduced a modified heart–lung machine that establishes postmortem circulation and allows for injection of a contrast agent and the constant perfusion of cadavers.[88] This technique was established for postmortem angiography to diagnose vascular lesions that could explain the cause of death and it has been further developed and standardized. Recently, Multiphase Postmortem Computed Tomography Angiography (MPMCTA) was introduced as a routine technique in several institutes of legal medicine.[90] The aim of this study was to adapt the system developed for MPMCTA to establish perfusion in human cadavers; our hypothesis was that this would facilitate endovascular procedures.

Materials and Methods

Subjects

The present study was performed on eight human bodies (six men and two women) donated by an anatomical institute. To preserve the anonymity of the donors, information such as medical history was not disclosed. All the donors were between 65 and 100 years old at the time of death. The study took place in the anatomy department of Freiburg University, Switzerland, in

three sessions between August 2010 and November 2011. The specimens were prepared according to the Thiel cadaver embalming technique.[38]

Postmortem CT

Four of the eight bodies underwent Postmortem Computed Tomography (PMCT) to detect calcification of the aortic valves. The examinations were performed with an eight-row CT unit (CT LightSpeed 8; GE Healthcare, Milwaukee, WI) with the following scan parameters: field of view, 50 cm; slice thickness, 2.5 mm; interval of reconstruction, 2 mm, 120 kV, 280 mA (modulated); and noise index, 15. The scan was performed from the cerebral vertex to the pubic symphysis.

Experimental Procedures

Each experiment was performed by experts from different disciplines who collaborated closely on each case. The experimental team comprised a forensic radiographer, who was responsible for the preparation and postmortem perfusion of the body; an interventional cardiologist, who was responsible for the delivery of the aortic valve and was assisted by an engineer responsible for developing the delivery system and preparing loading procedures for the transcatheter heart valve using the 18-Fr transfemoral delivery system; and a forensic pathologist, who opened the thoracic cavity and extracted the heart.

Preparation of the body and establishment of postmortem circulation

Throughout the procedure, the bodies lay on an X-ray-compatible table. An incision of approximately 10 cm was made in the right inguinal region. Then, using surgical tools, a path to the femoral artery and vein was opened. Once each vessel has been identified and cleared from the surrounding tissue, an 18-Fr cannula (Fumedica AG, Muri, Switzerland) was inserted into both the femoral artery and vein and tightened with strings and clamps. The tubing set of the perfusion device (Virtangio; Fumedica AG) was then connected to the arterial cannula to perfuse only the arterial system. The cannula in the vein was simply connected to the reflow bag to collect the perfusion liquid draining from the body. The final positions of the cannulas are shown in Figure 1.

Because the tested aortic valves were only expandable at a physiological body temperature of about 37°C, the body had to be warmed. Therefore, the injected perfusion liquid, composed of PL, had to be heated. For this purpose it was poured into a metallic bowl that was set on a camping hot plate and placed under the perfusion device (Fig. 2). Owing to loss of heat inside the tubing system, the liquid had to be heated to 50°C to attain a temperature of about 37°C inside the body. The initial perfusion began with a volume of about 500 mL, injected at a flow rate of 500 mL/min. Further injections could be performed subsequently when needed. The flow rate was manually adapted to the anatomy of the vessels (higher flow rate to bypass stenosis)

in accordance with the manipulations performed during the minimally invasive delivery of the aortic valve.

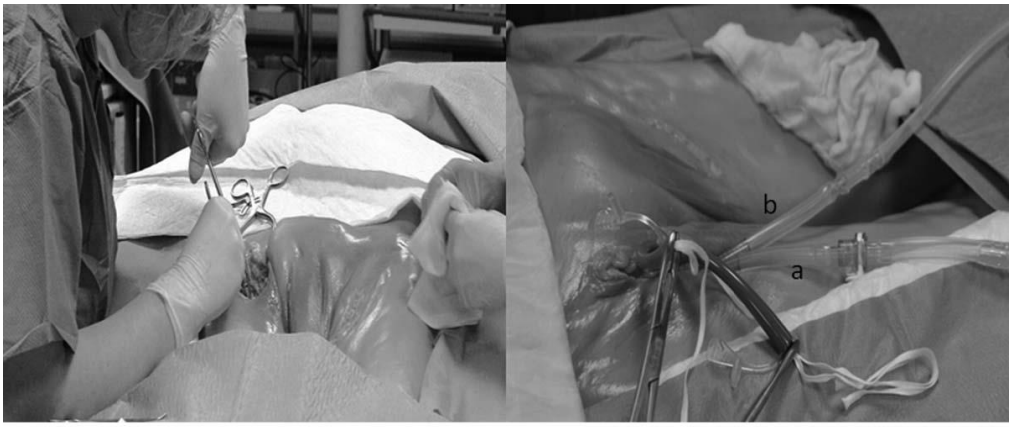

Figure 1. *Preparation of the right femoral artery and vein (A) and arterial (a) and venous (b) tubes fixed inside the vessels (B).*

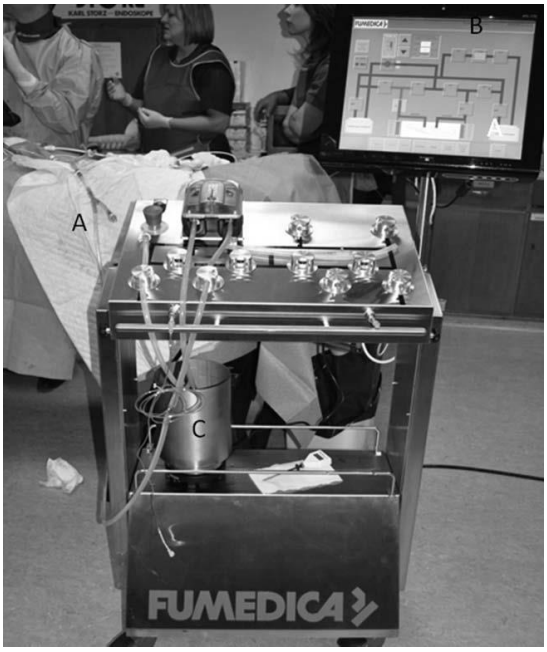

Figure 2. *Set-up for establishing postmortem perfusion. A Virtangio perfusion device was prepared for the experiment: (A) tubing set mounted on the perfusion device consisting of one single tube for the arterial perfusion. (B) Control screen with perfusion parameters. (C) Hot plate with metallic bowl containing paraffin oil, which was heated before it was injected into the arterial system.*

Preparation and delivery of the aortic valve

The transcatheter heart valve was rinsed to wash out the glutaraldehyde preservative and inserted inside a funnel, which eased the positioning of the connecting T bars to the catheter-loading anchor. After the connection, the bioprosthesis was pulled inside the retaining 18-Fr catheter until it was completely loaded. After its flushing ports had been flushed with 0.9 % sodium chloride, the catheter was ready to be introduced.

For the delivery, a sheath and a dilator catheter were introduced over the wire in the femoral artery, creating the vascular access. In all cases, catheterization was performed via the artery on the contralateral side of the access site chosen for establishing postmortem circulation. The entire endovascular procedure was monitored radiologically with a mobile fluoroscopy system (Philips BV Pulsera; Philips Medical system, Eindhoven, The Netherlands). The setup is shown in Figure 3.

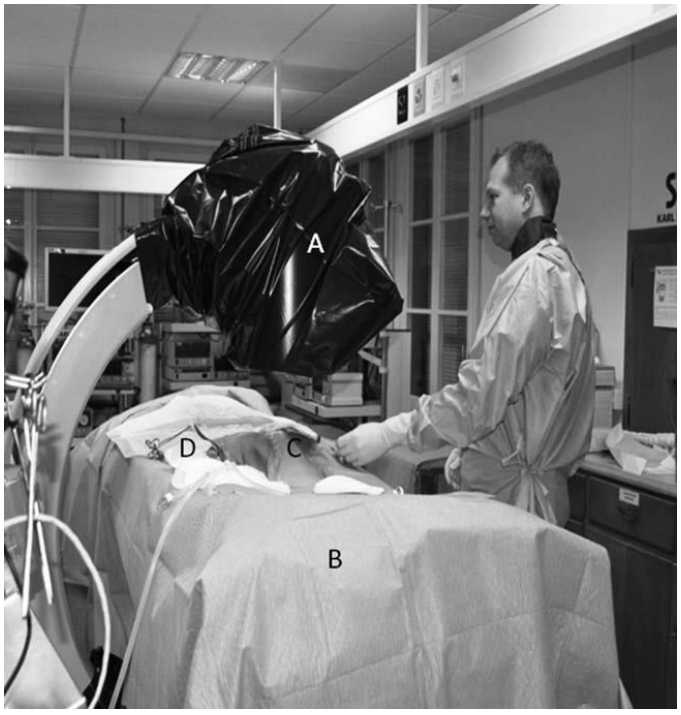

Figure 3. *Set-up for delivery of the aortic valve. (A) Fluoroscopic amplifier (with plastic cover protection) to control intravascular navigation. (B) X-ray-compatible table with the body in dorsal position. (C) Clinical access for endovascular intervention in the left inguinal region. (D) Access for postmortem circulation in the right inguinal region*

The left ventricle was catheterized with a soft guidewire and then an exchange maneuver was performed (Fig. 4A). In this technique, used in endovascular practices, one guidewire is exchanged for another. A compatible angiographic catheter is placed over the positioned guidewire and maintained at the catheterized site while the guidewire is withdrawn. The new guidewire is subsequently introduced into the catheter lumen and finally the catheter is withdrawn from the wire, which replaces the anterior guidewire. This maneuver allows for

exchange of the existing wire without loss of the catheterized position. Once the left ventricle was accessed, a superstiff guidewire was used to maintain a secure position within the left ventricle. Using an over-the-wire device, a valvuloplasty balloon was placed at the level of the native valve and insufflated, thereby expanding the calcified valve (Fig. 4B). The balloon was then deflated and withdrawn. A 5-Fr introducer sheath and dilator catheter was placed in the right femoral artery; then a 5-Fr pigtail catheter was introduced into it and placed directly in the aortic sinus behind the noncoronary leaflet. A pigtail catheter was used for controlled injection of the contrast agent Angiofil® until the aortic root showed a contrast “shadow,” which allowed its structure to be seen without obscuring the endovascular devices. The pigtail catheter loop marked the position of the aortic annulus. The 18-Fr delivery system (New Valve Technology), with the prosthetic aortic valve loaded on to it, was placed over the superstiff guidewire; under fluoroscopic control, it was pushed toward the native aortic valve (Fig. 4C). The prosthetic valve was deployed within the aortic annulus over the native leaflets (Fig.4D). During deployment, the temperature of the paraffin solution was kept above 37°C to allow the NITINOL stent (a nickel–titanium alloy distinguished from other materials by its shape memory and superelastic characteristics) to recover its thermomemory shape. The delivery position of the valve in relation to the annulus, based on the angiographic view and all of the implantation characteristics were verified by fluoroscopy and recorded (Fig. 5).

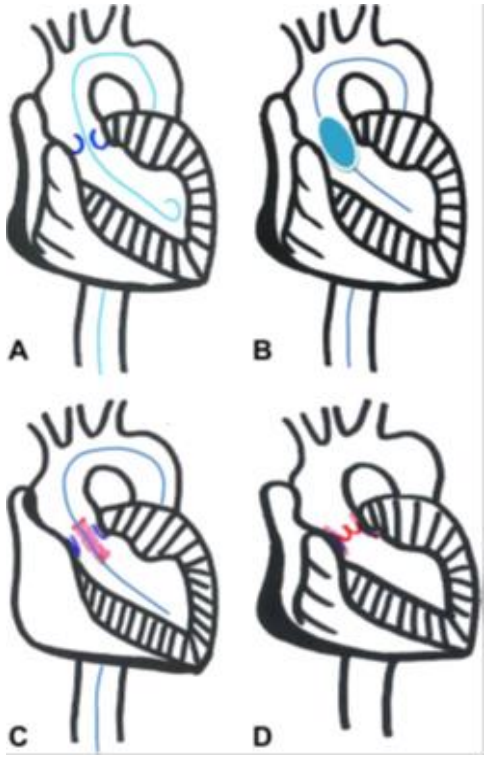

Figure 4. (**A–D**) *Schematic drawing of the procedure for a transcatheter aortic valve replacement.*

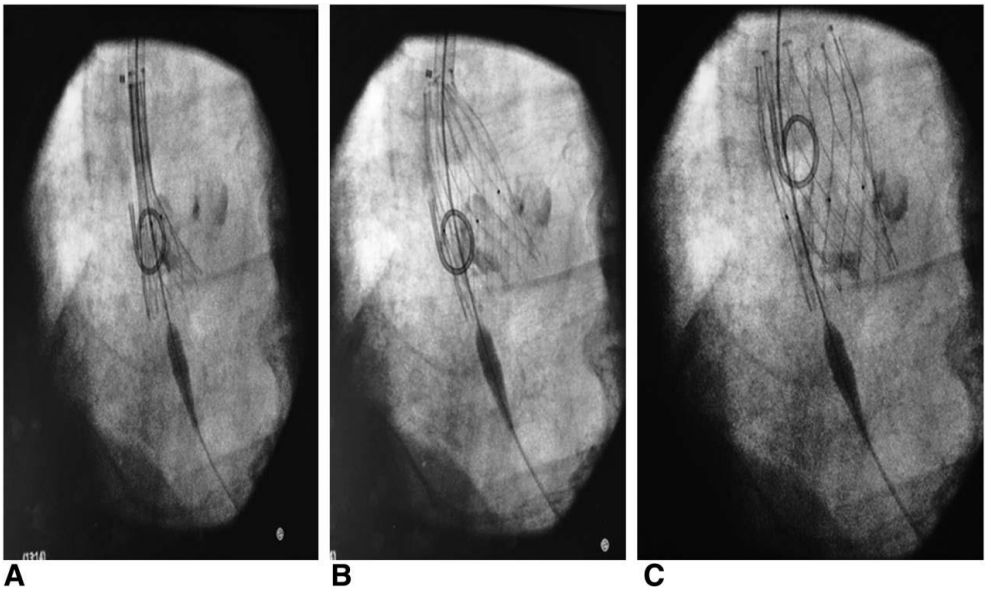

Figure 5. *Delivery of the aortic valve under X-ray control. Once the material was delivered, the self-expanding aortic valve took its original form (A–C).*

Quality control of postmortem perfusion

The experimental team insured quality control of the postmortem perfusion using a three-step scale: filling of the vascular system was considered to be good if the perfusion was done optimally in one step (no refill needed), average if the perfusion had to be executed in two or three steps (one or two refills needed) and poor if the perfusion had to be performed in several steps (multiple refills necessary).

Control of positioning

To assess the correct positioning of the implanted aortic valve, the thoracic cavity was opened according to standard autopsy guidelines (Recommendation no R (99)3, 1999) using a medial linear incision.[209] The pericardium was opened and the heart was extracted. Special attention was paid to insuring that the ascending aorta and the aortic arch were extracted together with the heart. The aspect and relative position of the prosthetic aortic valve to the valsalva sinus, the compression of the native leaflets and the calcified structure and its influence on the patency of the coronary ostia, were observed and registered (Fig. 5). In addition, the presence or absence of calcifications on the aortic valve was determined.

Results

Postmortem CT

None of the four cases investigated by PMCT showed calcifications on the aortic valve.

Establishment of postmortem circulation and delivery of the aortic valve

Filling of the arterial system was good in seven of the cadavers; however, the eighth cadaver was only moderately filled. In this case, huge arteriosclerotic plaques were observed in the abdominal aorta, which created a significant stenosis of the lumen. After refilling of the eighth cadaver, all of the cadavers were perfused, which was one of the research goals (Fig. 6).

Figure 6. *Control of the position of the delivered aortic valve. Superior view of the aortic valve after extraction of the heart.*

Discussion

The present study describes a new human model for testing endovascularly delivered material. We tested this model using eight cadavers that had been embalmed by the Thiel method.

Ideal testing of endovascular procedures in human cadavers requires leak-proof, long-term circulation; arterial pulsation; and visible filling of the major vessels. In 2001, Garrett described pump-induced circulation in several isolated common arterial circuits of fresh human cadavers.[97] This model offers realistic conditions for training clinicians in several endovascular techniques such as balloon angioplasties, expansion of stents and stented grafts and for investigating new devices throughout the arterial tree. Despite these merits, the absence of reperfusion of the venous system and the development of edema were significant limitations. In 2009, a similar “warm” human cadaver model was developed for testing endovascular procedures of the thoracic aorta.[176] This model was useful but extravascular leakage was a significant finding. Recently, Aboud and Moursi reported a more lifelike reperfused human training model that comprised both vascular systems, enabling training and testing procedures

on the heart and major vessels.[96] In this model, a machine provided pulsating pressure in the arteries, while constant pressure was generated in the veins. A drawback was separation of the two circulations, such that no arteriovenous circulation existed. Moreover, revascularization was limited to isolated vessel segments.

Experiments to establish vascular perfusion of cadavers have also been carried out to visualize the vessels of the deceased to determine the cause of death. Dynamic angiographic analyses of a whole body were initiated to explore the vascular system for this purpose. The concept of these experiments was that to perform PMCT angiography that would closely resemble clinical angiography, conditions would need to be similar to those *in vivo*. As a result, the idea arose of establishing a "postmortem circulation" that would allow for perfusion of the body.[210] A first feasibility study, performed on an animal model, demonstrated that the concept was successful; diesel oil was used as the perfusate and was circulated through a roller pump.[87] An oily perfusate was chosen because oily liquids stay within the vascular system[211], which makes them suitable for initiating perfusion without large losses of the perfusate into the surrounding tissue, which would cause edema[212]. After successful application of the concept to an animal model, the first trials were conducted to adapt the technique to a human model. Two essential changes were made: the perfusion device was changed from a roller pump to a modified heart–lung machine and the diesel oil was replaced by the odorless oil PP.[88] The images obtained revealed the vascular anatomy in detail, up to the level of arterioles. However, a major problem with the technique was a discharge of the perfusate into the stomach and the intestine; this was found to be due to a locus minoris resistentiae.[87] This finding was not surprising, given the combination of bacterial decomposition and autolytic activities in the gastrointestinal tract, which can lead to an early increase in vascular permeability in this region. To overcome this problem, the perfusate was later changed from PP to the more viscous PL.[90] Additionally, useful materials for performing postmortem angiography have been developed recently, including a specialized and easy-to handle perfusion device and a single-use set that contains a tubing set and oily contrast agent.

The present study describes the use of technology that was originally developed and used for forensic purposes in clinical anatomy. However, our findings suggest that it is also a useful tool for testing medical devices and materials, because it provides a model in which the conditions more closely resemble those found in living patients than the currently used animal and virtual models. To our knowledge, this is the first time this technology has been used for such a purpose.

Our experience with this approach demonstrates that, with good filling of the vessels, the placement of new materials and the training of clinicians in their delivery and use is facilitated by perfusion of the cadavers. This is because in most human cadavers, the vascular system is collapsed (Fig. 7A), which hinders the passage of endovascular catheters within blood vessels.

By performing postmortem perfusion, thus filling the vessel with perfusate or a contrast agent, the vascular lumen is reopened (Fig. 7B) and endovascular navigation is enabled. A retrograde constant flow of the perfusate, supplied by the perfusion device, allows even severe stenoses to be bypassed; these would represent impassible barriers without the perfusion technique. In fact, our first experiments, performed by the same clinical team but without the presence of the postmortem team and without postmortem circulation, had to be abandoned. These failures motivated the collaboration between clinical and postmortem disciplines and also the initiation of the present study.

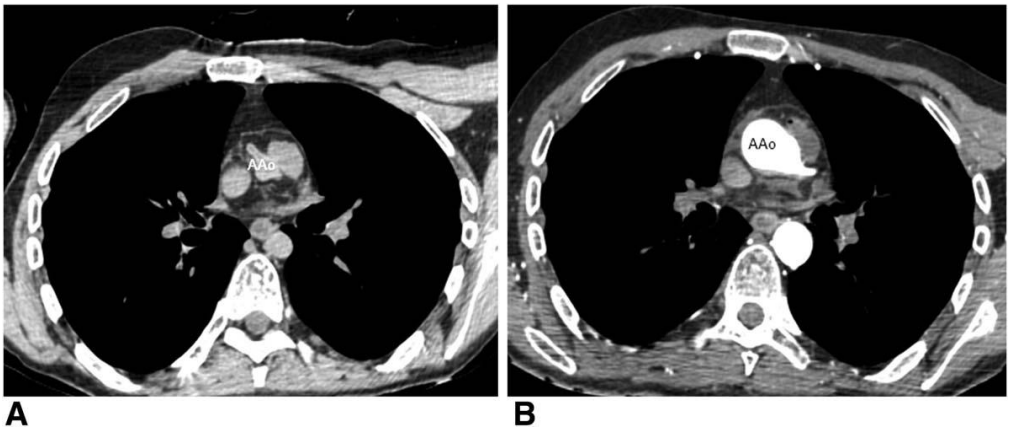

Figure 7. *Effect of postmortem perfusion on the vascular system. (**A**) Unenhanced CT scan revealing collapsed ascending aorta (AAo). (**B**) Arterial phase of CT angiography showing the AAo expanded and filled with contrast agent (white).*

Although this new approach demonstrably allows for easy endoluminal navigation and delivery of the material under investigation, there are some difficulties not encountered in real clinical conditions. It was more difficult to control injections of the contrast agent than in living patients, because the contrast agent was not completely washed out of the aortic root and arch. This could be due to the retrograde flow, or to the high viscosity of the injected contrast agent Angiofil®. Because the contrast agent is an oily liquid, this disadvantage could be overcome by simply decreasing its viscosity. This is done, for example, to perform microangiography.[88, 213]

Our experience also highlights the advantages of combining clinical and postmortem specialists. This collaboration allowed for direct *in situ* control of the implanted material without time loss, as well as fruitful interdisciplinary exchanges.

We realize that performing a native CT scan before the test procedure can help to choose those cadavers that would best match the needs of the experiment, as many medical materials are specifically developed for defined pathologies. Unfortunately, in this study, none of the radiologically screened bodies had a calcified aortic valve, which would have been very useful for our experiments.

In conclusion, the results of our experimental study reveal that the postmortem circulation model could be useful for testing endovascular material and implantation techniques. Delivery of the aortic valve system that we tested was possible with this model. Handling and tracking of the catheters was successful; all of the tested implants easily reached the implantation site, due to expansion of the vascular system and the intraluminal flow created by postmortem perfusion. The anatomical apposition of the implant with the human aortic root, its placement at the correct level within the aortic anatomy and any interference with the coronary ostia and with the mitral valve anterior leaflet, were detectable with this new technology. This model could represent a step forward in preclinical assessments during the development of transcatheter valves and other types of endovascular devices.

Chapter 12

Discussion

The following study's key findings are summarized that form the basis of this thesis:

1. The effectiveness of non-reperfused human cadavers to gain laparoscopic dexterity has been poorly studied. **(Part A)**
2. Various reperfused human cadaver models exist, but a long dynamically reperfused model has not yet been reported. The cadavers' preservation method determines the level of simulation fidelity. Thorough evaluation as a surgical training tool has not been done. **(Part B)**
3. PP flows from artery to vein in fresh pig tissues under acceptable vascular conditions, but with considerable leakage. **(Part C)**
4. Arterially injected Thiel embalming solution is thin and completely spreads in pig kidneys and results in successful preservation. PP runs for several hours in Thiel embalmed pig kidneys without increasing weight, volume and pressure. **(Part D)**
5. Early and quick postmortem pump-driven embalming in the pig model enables PP to flow for a long time from artery to vein and to enter the capillaries of the small intestines. Leakage that varies between the organs remains without major impacts on tissues' gross anatomy and suitability for surgical training. **(Part E)**
6. PP circulates for hours from artery to vein in isolated human organs and one arm and avoids gross anatomical deformations due to low established vascular pressures. Effective venous drainage in a complete human body occurs locally, indicating that vascular reperfusion differs between body parts. **(Part F)**
7. Aortic pressurization in Thiel cadavers enables endovascular aortic valve delivery. **(Part G)**

PART A: Systematic review of laparoscopic skills training.

Intuitively, human cadavers seem to be the best clinical-like models for advanced surgical procedural training. Surprisingly, their role within the context of laparoscopic surgical skills training is limited and their benefit remains unknown **(Chapter 4)**. Several reasons for their minor role can be put forward: availability limited to anatomical centers, long preparation, often high costs, need for operating table and only available to perform advanced surgical skills training.[104] Other training models like VR and VT are more popular due to their availability, reusability, easier transport and the possibility to train more and more advanced surgical skills. Moreover, VR simulators automatically register parameters that can be used for objective assessment. As a result, in contrast with human cadavers, these training tools have been extensively analyzed in comparative trials. Gilbody et al. underline our findings that human cadaver surgical training is poorly studied with only eight studies reporting their use in workshops.[7] Well designed studies are necessary since only three trials have objectively assessed trainees' performance during cadaver training and only two publications evaluated skills transfer to real life. Nevertheless, trainees and tutors value human cadaver training to

enhance surgical skills. Additionally, this review highlights the difficulty in designing and interpreting educational studies, especially to proof skills transfer to real life.

PART B: Systematic review of reperfused human cadavers and body parts.

The wide range of techniques to reperfuse human cadaver models implies and emphasizes that filling the vascular system is complex. **Chapter 5** is the first systematic review that describes these advanced surgical training models. This review can be a useful basis for further elaboration of reperfused human cadaver models. Certainly, besides creating new applications, researchers intend to enhance the realism of the surgical exercises by using lifelike tissue preservation methods such as the Thiel embalming technique and by creating vascular reperfusion.[36, 37, 39-44, 46-48, 52, 55, 56, 60, 61] Surprisingly, surgical training on reperfused Thiel cadavers is limited to three studies, whose reperfusion set-up is relatively simple.[28, 59, 91] Bouma et al. cannulated the left ventricle of Thiel cadavers, which was then pressurized with saline solution and blood through a pulsatile intra-aortic balloon pump.[28] This model allowed trainees to perform coronary artery bypass grafting. Chevallier et al. cannulated the femoral artery of Thiel cadavers and then retrogradely filled the aorta with viscous PL, permitting endovascular delivery of aortic valves **(chapter 11)**.[91] Wolff et al. employed Thiel cadavers and installed a pulsatile flow with red tap water in the arteries of prelevated flaps.[59] Note that fresh cadavers are a valuable alternative, but their usage is short, impeding the execution of multiple workshops on one cadaver.

Combined, although these models may have their merits, a Thiel embalmed surgical training model with a long dynamic flow - including all arteries and veins - using a proper perfusate like we intended to develop through this thesis has not been described in literature. Note that this model is particularly useful for a wide extent of advanced surgical procedures for different surgical disciplines. Moreover, sufficient venous filling creates new surgical opportunities and due to the presence of a vascular flow bleedings are more notable.

Although we intend to increase the level of simulation fidelity of this model, it is important to mention that the curriculum in which it is implemented combined with qualitative performance feedback are at least of similar importance. Thus, this reperfused model must be implemented in a training curriculum. However, practice is only useful if a competency-based VR training curriculum for the acquisition of skills has been followed; if at least basic skills have been acquired; and if some cases in patients have been assisted or performed. In addition, as mentioned above in Part A, high quality assessment of reperfused cadavers with standardized assessment instruments and subsequent evaluation in the patient are needed to explore the real value of this model within the extensive range of surgical training models.

PART C: Exploration and assessment of dynamic reperfusion in fresh pig tissues: two pilot studies.

Trying to develop a long dynamic reperfusion in Thiel human cadavers is an ambitious project and was initiated by working in an animal model. Although pigs have smaller vessels than humans, these animals were chosen because of their close adherence to human anatomy in contrast to dogs and cats.[87] From the beginning, an oily liquid was the preferred perfusate as it remains longer within the vessels.[82] Therefore, viscous PL was employed during initial experiments in the hindquarter and lower body of a fresh pig. However, time until venous drainage was enormously long because the installed low flow rates were associated with too high arterial pressures (unpublished observations). Hence, thinner PP was used that has proven its efficacy during short (*i.e.* five min) dynamic reperfusion in fresh human extremities without causing any edema, possibly because of short reperfusion time and the low arterial pressure (50 mmHg).[88] Therefore, our two pilot studies **(chapter 6 and 7)** are unique because longer circulation in fresh tissues has never been analyzed in terms of arterial pressure/flow rate - time relationship, volume loss and perfusate distribution. Szinicz et al. also successfully installed prolonged circulations, but with tap water under high arterial pressures (150 - 180 mmHg) in fresh pig organs. In this research project, watery solutions were not chosen due to the fast occurrence of extravasation, causing tissue deformation. Although leakage occurred in our experiments, we preferred PP based on acceptable generated arterial pressures - limiting vessel wall damage - and preserved gross anatomy.

In a next experiment, we reperfused a fresh pig of 33 kg. Under general anesthesia, the common carotid artery and external jugular vein were cannulated. Then, 45,000 units of heparin were administered intravenously to prevent blood clots and to facilitate reperfusion with PP. Afterwards, euthanasia was performed with T61. PP mixed with 6 % Angiofil® was injected with the pump in the arterial cannula. This flow was continuously visualized on CT. Venous drainage occurred and an ongoing circulation (flow rate of 350 mL/min and arterial pressure of 120-130 mmHg) was feasible for 30 minutes. In total, 5 L PP was injected, of which 1.9 L was lost. CT showed initial nice filling of the major vessels without extravasation. Gradually, accumulation of PP in the lungs, liver and mesentery was noted. A laparotomy showed limited intraperitoneal accumulation of PP. The mesenterial vessels were almost completely filled with blood, obstructing inflow. The liver was pale and had no soft constitution. Incision of this organ resulted in diffuse drainage of PP. The space of Disse was expanded on microscopic imaging. Similarly, widened alveoli were found, indicating lung edema. Also, the space between muscle fibers in the heart and extremities was wider due to accumulation of PP in the interstitium. This experiment demonstrated that PP flows in a complete pig body from artery to vein, but leakage remained.

PART D: The spread of Thiel embalming solution in pig kidneys and subsequent long dynamic reperfusion of these embalmed organs.

In a next step, the flow of PP was evaluated in two Thiel embalmed pigs. An identical experimental set-up as described in the previous experiment was then installed, but a flow from

artery to vein was not realized in the first Thiel embalmed pig. Probably, accumulated embalming fluid hindered reperfusion, but autopsy also showed local insufficient embalming. In the second pig we did not establish a reperfusion with PP because moderate embalming was again found on laparoscopy and thoracoscopy. We assumed that accumulation of embalming fluid in the tissues may block subsequent reperfusion with PP. Therefore, as a test, the second pig was immersed in a concentrated salt solution (4 kg salt in 20 L tap water) for 18 days which caused a weight loss of 3.5 kg.

This weight loss procedure was then elaborated in multiple Thiel embalmed goat and pig kidneys. These embalmed kidneys were immersed in varied concentrated salt solutions for several days, during which at regular times weight loss was measured. Eventually, immersion in concentrated salt solution of 0.300 kg salt/L tap water for seven days caused sufficient and a steady state weight loss. The amount of salt (kg) was the same as the weight of the embalmed kidney.

Because reperfusion of a Thiel embalmed pig was not possible, a reorientation toward a simpler model was thus necessary. Reperfusing Thiel embalmed pig kidneys was a logic next step. Kidneys were chosen because of their small calibre and easy control of the hilar vessels. However, exploration of the renal distribution of the embalming solution first needed to be studied **(chapter 8)**. CT-graphic analysis of pump-induced spread of Thiel embalming solution in tissues has never been performed. Moreover, in a simple kidney model with patent vessels, we demonstrated that sole arterial embalming with a pump is sufficient, hence making postfixation by immersion superfluous. Whether these findings can be extrapolated to a complete human cadaver model remains to be investigated.

Reperfusing embalmed organs differs from fresh specimens in terms of weight gain - causing vascular narrowing - and tissue fixation. Eisma et al. stated that embalmed cadavers gradually lose their fluid excess during storage.[37] Immersion of Thiel embalmed pig kidneys in concentrated salt solutions accelerated this process, theoretically facilitating later vascular reperfusion. Probably, dehydration will only be necessary if reperfusion of a complete Thiel human body is intended in the immediate phase after immersion in the embalming bath. In contrast with our previous experiments in fresh pig tissue, we demonstrated that long reperfusion with PP leads to a steady state situation in terms of generated vascular pressure, weight and volume. Moreover, although only indirectly proven through glomerular dilatation, we confirmed the capillary flow of PP in Thiel embalmed tissues.

PART E: Long dynamic Thiel embalmed pig reperfusion, evaluation of the microscopic vessel recruitment and feasibility for surgical practice. A plea for pump-driven embalming.

Direct euthanasia combined with pump-driven and pressure-controlled embalming creates ideal circumstances for vascular reperfusion in a pig model **(chapter 9)**. Although researchers inject Thiel solution with a pump, none have stressed the importance of pressure measurement during embalming and have demonstrated capillary patency after pump-embalming, suggesting that pumps facilitate capillary rinsing.[37, 38] Note that in human cadavers, other factors such as microvascular patency (*e.g.* in case of atherosclerosis) and time until embalming, causing progressive capillary wall permeability due to tissue degeneration, may affect capillary rinsing with a pump.

In the previous experiment **(chapter 8)**, the capillary flow of PP was indirectly proven. However, here, we visualized the dynamic capillary flow of PP with DiI, a lipophilic carbocyanine dye that merges into endothelial cell membranes upon contact. This product has been successfully used in a specially formulated aqueous solution to visualize blood vessels in small experimental animals.[203] Note that DiI was only soluble in PP after adding 99.8 % alcohol to this product. Similarly, Grabherr et al. mixed diesel oil with Fluorol, a fat-soluble fluorescent agent (2, 8-dimethylnaphtho[3, 2, 1-k]xanthene) and administered this mixture into the vasculature of chicken embryos.[87] The author found that diesel oil does not enter the capillary network, proving its higher viscosity than PP. We preferred DiI because of practical reasons: sodium fluoride production has been discontinued (Sigma-Aldrich) and its fluorescent emission is not captured by our filter sets.

Inter-organ leakage variation during vascular reperfusion has been reported. Bruguier et al. indexed and identified possible artifacts related to MPMCTA in fresh human cadavers.[205] Therefore, firstly PL was retrogradely injected in the femoral artery. This was followed by antegrade administration in the femoral vein. Lastly, a dynamic (circulating) phase was performed for 150 seconds. Like in our study, for example, the prevalence of gastrointestinal mucosa artifacts (mucosa enhancement (i.e. increased mucosal contrast agent uptake), edema, extravasation) differed among stomach, duodenum and colon, but generally grew slightly with an increasing number of effected phases. Although viscous PL and short reperfusion in fresh cadavers were employed, leakage is unavoidable. The same authors did, however, not state whether this short reperfusion had an impact on the gross anatomy.

Fluid movement across capillary walls is the result of three processes (*i.e.* diffusion, filtration - formulated in Starling's equation - and pinocytosis). Only the hydrostatic capillary and interstitial pressures as part of Starling's equation remain valid during capillary reperfusion of embalmed tissues using osmotically inert PP. In the living, blood pressure significantly drops in the capillaries due to their huge total cross-sectional area. In Thiel tissues, we do not know to which extent capillaries are reperfused. Hence, pressure drop at this level is unknown. Certainly, establishing low pressures and using a perfusate with a proper viscosity is of paramount

importance to limit paracellular extravasation in the capillaries. Of note, whether PP enters the lymphatic vessels is unknown.

We demonstrate in this reperfused Thiel embalmed pig model that a wide range of surgeries can be practiced. Particularly, repeated training on organs (*e.g.* liver, intestines, stomach, kidney, spleen, lungs, etc.) for several hours without significant tissue deformation opens new perspectives. Only Aboud et al. offered similar training in the context of trauma surgery. However, formalin-fixed cadavers' stiffness and brownish discoloration, the need to make arteriovenous shunts prior to install a flow and the use of thin water that quickly causes deformations are important limitations in terms of reality simulation.[95]

PART F: Preliminary results of long dynamic Thiel embalmed human tissue reperfusion.

The experience in the pig model helped us to perform and interpret vascular reperfusion in Thiel embalmed human tissues **(chapter 10)**. Unexpectedly, in human tissues, long dynamic reperfusion is more complex and differs significantly from the Thiel embalmed pig model. Initially, we assumed that installing a flow in human tissues would be much easier due to their solidity since pigs have more fragile tissues (personal observation). However, these reperfused human organs were generally prelevated from cadavers with significant vascular disease that had been stored for approximately one year. Moreover, these bodies were embalmed by gravity. Gravity-embalming takes longer than pump-embalming and thus may result in lower established capillary pressures, less capillary rinsing, lower degree of tissue penetration, ongoing tissue decomposition and thus inferior embalming quality. For example, capillary obstruction was observed in gravity-embalmed human intestines. Another example, demonstrating the difference between human and pig cadavers was noted in kidneys. Despite similar arterial pressures in both models, more time was needed in human kidneys (*i.e.* two hours in human vs. one hour in pig) until no further weight gain was observed. In addition, PP entered the human ureter. Both findings indicate that the capillary wall was more obviously passed. Ureteral filling was absent in pig kidneys, demonstrating better microscopic tissue preservation. A last example demonstrating the difference between humans and pigs was found in the complete human cadaver: in contrast with the embalmed pig model **(chapter 9)** no drainage in the venous cannula occurred after arterial administration of PP, but local venous drainage was present. This underlines that the flow of PP depends on embalming-related factors like the quality of embalmed tissues (*i.e.* termination of capillary wall permeability increase due to tissue decomposition) and capillary patency, which depends on the extent to which the embalming solution rinsed the vessel lumen. Also, the placement of a venous cannula during embalming is essential to permit drainage of blood, facilitating venous outflow of PP. Besides, it is important to mention that a higher arterial pressure than in the living must be avoided during the flow of PP, limiting unnecessary vascular wall damage. Finally, local anatomy contributes to the effective flow of PP. Vessel wall permeability, for instance, rapidly increases in the postmortem phase in the gastro-intestinal tract due to a combination of

bacterial decomposition and autolytic processes.[87] Moreover capillary type (*i.e.* continuous vs. fenestrated vs. sinusoid) and presence of metarterioles, which connect arterioles and venules determine the local reperfusion of PP.

Note that this type of flow in Thiel embalmed human organs/cadaver has never been established **(chapter 5)**. Grabherr et al. installed a short dynamic flow of 150 seconds in fresh human cadavers, but realized this only after the arterial and venous systems were separately reperfused.[90]

PART G: Arterially pressurized Thiel embalmed cadavers: an essential model for training.

As demonstrated in **chapter 5**, basic to complex reperfused human models may allow training a variety of open and minimally invasive surgical techniques. In **Chapter 11**, we reported that arterial pressurization in Thiel cadavers enables endovascular aortic valve deployment via the transfemoral route and that this is a potential model for advanced endovascular training. Reperfusion is essential, as in initial experiments no arterial filling was present and the collapsed vessels hindered effective introduction and accurate placement of the aortic valve. None of the current animal models under general anesthesia allow correct evaluation of the aortic valve deployment, position, anchoring and functioning in the orthotopic position due to considerable anatomical limitations and differences compared with humans.[208]

A drawback of this thesis is that its hypothesis - is it possible to reperfuse a complete Thiel embalmed body - was from the beginning very ambitious. Moreover, neither experience nor knowledge about vascular reperfusion was available, forcing us to start with experiments on fresh animals. Although essential, substantial research time was spent on fresh and embalmed animals with patent vessels, during which we expected that the step to extrapolate our results to human specimens would be trivial. However, vascular patency in Thiel human cadavers varies substantially and is never perfect. Additionally, we gradually learned that the Thiel embalming technique at our institution is imperfect, compelling us to investigate the technique and the solution's tissue spread. As a consequence, the hypothesis has only been fulfilled in one cadaver. The biggest challenge, which is thorough investigation and improvement of the reperfusion process in complete Thiel cadavers has not yet been done, but can be performed in the future.

In summary, (non)-reperfused human cadavers' intrinsic value as a surgical training model remains unknown. PP runs from artery to vein through the capillary network in pump-driven pressure-controlled Thiel embalmed pig models for a long time under acceptable intravascular conditions, but with leakage that varies among organs. This model, however, has no major gross anatomical effects, enabling realistic simulation of various surgical procedures. Pump-embalming is indispensable to facilitate vascular reperfusion. PP also circulates in gravity-embalmed Thiel human kidneys, one liver and one arm under permitted intravascular

circumstances and has acceptable gross tissue effects, but its microvascular behavior in these human tissues has not yet been rendered visible. Although preliminary, in one complete Thiel cadaver, PP locally enters the veins, but drainage in the venous cannula is absent, underscoring that elaboration in a total human model is needed.

Chapter 13

Future perspectives

This reperfused model opens up many new perspectives for anatomical research, experimental surgery and education.

Anatomical research

This research mainly focuses on enhancing the vascular embalming procedure. As repeatedly mentioned in this thesis, we assume that pump embalming is the preferred technique. To support this statement, a comparative pilot study is in progress to investigate the spread of arterially administered Thiel embalming solution in human cadavers. Firstly, fresh cadavers undergo a whole body CT-scan. Then, arterial embalming is done by gravity in one group. In the other group, the embalming solution is injected with a pump. A cannula is also placed in the femoral vein, allowing drainage of excess venous blood. In each case, embalming volume, embalming time and postmortem delay until embalming will be collected. After vascular embalming, a new CT-scan of the bodies will be performed. The purpose is to assess the solution's intracorporeal spread in Hounsfield Units on the CT. If beneficial effects are confirmed after pump-embalming, this technique will be implemented in future embalming procedures. Moreover, if we can CT graphically demonstrate that the skin and subcutaneous tissues are sufficiently embalmed, immersion in a Thiel bath may become redundant.

Postmortem accumulated blood and clots may block vascular spread of embalming solution and PP. Hence, a vascular rinsing solution will be pilot-tested before vascular embalming is initiated. Similar to the previous study, CT-scans of fresh cadavers will be performed; then, on-pump injection of histidine-tryptophan-ketoglutarate will be started via the femoral artery until clear fluid drains from the venous cannula. This product is effective to wash-out organs prior to transplantation.[214] Finally on-pump embalming will be carried out followed by CT scanning. The embalming fluid's spread will be compared with the CT-graphic results of the bodies in the previous experiment.

In addition, we will try to accomplish that embalming is done under fixed approximately lifelike arterial pressures, improving tissue perfusion and intravascular wash-out, but without causing any vascular damage. Therefore, engineers are currently converting our flow-rate-driven pump to a pressure-driven device.

At the Department of Anatomy in Ghent, we still employ the original perfusion solution 1989 and infuse 15.8 L perfusion fluid for an average size body.[39] Current insights, however, favor the infusion of slightly different formulae and perfusion volumes than the most recent version published by Thiel in 2002.[37] Thus, further aims are improvement of the embalmed tissue quality through implementation of these adjusted mixtures.

Tackling early postmortem tissue decomposition is a challenge. Perhaps, immediate postmortem wash-out of the stomach and colon with an isotonic solution followed by direct infusion with visceral solution – as originally reported by Thiel – may be advantageous for tissue

quality.[32] Moreover, this may limit perfusate leakage in the gastrointestinal tract during later vascular reperfusion.

Experimental surgery

Enabling and/or facilitating vascular reperfusion in Thiel embalmed organs, extremities and cadavers necessitates extensive research as reported above. However, while these experiments are running, vascular reperfusion can be examined in embalmed human body parts with known venous return. In spite of the excellent reperfusion properties of PP in Thiel embalmed human kidneys, significant leakage occurs in other organs. Consequently, an experiment will be commenced to analyze the vascular flow of several perfusates (*i.e.* PP, PL, PEG 200 and 400, etc.) in isolated Thiel body parts (*i.e.* liver, lungs, kidneys and extremities). Using a fixed arterial pressure, flow will be compared in terms of reperfusion time, flow rate, vessel reperfusion, microscopic and macroscopic tissue effects. Hopefully, the mentioned measures will aid in improving whole body reperfusion.
Additionally, minor adjustments of the reperfusion process are the development of a pulsatile arterial flow and circulating at body temperature.

Education

Generally, this training model is suitable to practice numerous surgical procedures. Below, some examples of training sessions are given, which could be offered with this model in the future.

Pulsatile organ perfusion

A proper perfusate that flows in Thiel embalmed human organs can ameliorate the model published by Szinicz.[86] This enhanced training tool permits to execute basic (*e.g.* CCE, triangle of Calot dissection, suturing, etc.) and more advanced surgical techniques (*e.g.* hepatic resection).

Figure 1. *Pulsatile organ perfusion model developed by Szinicz.*

Open vascular and plastic surgery

As illustrated in **chapter 10**, open vascular skills can be trained on reperfused isolated human extremities. Wolff et al. reported flap raising on pulsatile perfused cadaveric tissue using tap water, but the model described in this thesis may also be suitable to learn and practice arterial and venous microanastomoses.[59]

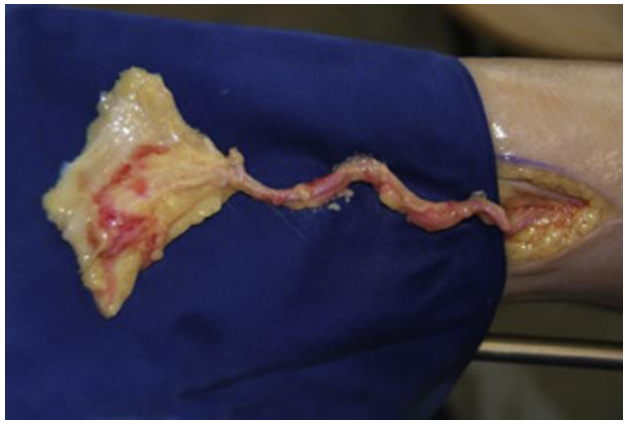

Figure 2. *Perfused radial forearm flap in an embalmed cadaver (adapted from Wolff et al.).*[59]

Laparoscopy and thoracoscopy

Although long dynamic reperfusion of a complete whole body with obvious venous drainage is still in a developmental phase, the described reperfused human cadaver method in **chapter 10** may be practical in any type of minimally invasive surgical procedure.

As stressed in **chapter 5**, validation of reperfused human cadavers and body parts is of paramount importance. However, research protocols are necessary prior to start any workshop. Validated OSATS derived objective structured assessment tools are needed to measure technical performances and to prove transfer of skills to the real patient.

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Appendix 1

Characteristics of included studies

Study	Participants	Interventions	Training tasks	Duration of training	Model used for assessment + task
Aggarwal *et al.*[99]	20 novices	VR vs no training	VR (easy + medium level): instrument navigation, grasping tissues and clip application	7 basic tasks: performed twice at the easy and medium levels	VT: laparoscopic CCE on a cadaveric porcine liver with gallbladder
Ahlberg *et al.*[117]	29 medical students	VR vs no training	VR: 6 tasks	3 h (trained twice)	Laparoscopic pig model: simulated appendectomy
Ahlberg *et al.*[118]	13 PGY1-2 surgical residents	VR vs no training	VR (3 levels of difficulty): suturing with and without easy grip function, lift and grasp, clipping and ultrasonographic dissection	1 h sessions with a maximum of 8 sessions per day for 1 wk till proficiency	10 laparoscopic CCE on patients within 6 months after training
Calatayud *et al.*[10]	10 surgeons	VR vs no training	VR (medium level): lifting and grasping, clip applying and dissection	VR: 5 subjects: training (15 min) directly before laparoscopic CCE followed by a 2nd laparoscopic CCE with no training. 5 subjects: laparoscopic CCE without training, followed by a 2nd laparoscopic CCE with preprocedural training	2 laparoscopic CCE on patients (2 wks between each case)
Grantcharov *et al.*[8]	20 surgical residents	VR vs no training	VR: grasp and place, grasp transfer and place, tranversal, withdraw and insert, diathermy, manipulate and diathermy	VR: 10 repetitions of the tasks	Partial laparoscopic CCE on a patient (from clipping till removal of the gallbladder within 14 days after start experiment
Hogle *et al.*[113]	13 PGY1 surgical residents	VR vs no training	VR: camera and instrument navigation, coordination, grasping, lifting and grasping, cutting and clip applying	VR: at least 2 sessions per wk	2 elective laparoscopic CCE on patients
Hogle *et al.*[113]	21 surgical residents	VR vs no training	VR: camera and instrument navigation, camera–instrument coordination, grasping, lifting and grasping, cutting and clip applying	VR: for 5 wks till proficiency	At the end of the 5-wk period : laparoscopic CCE on pig model

Study	Participants	Interventions	Training tasks	Duration of training	Model used for assessment + task
Hyltander *et al.*[119]	24 medical students	VR vs no training	VR: hit balls that appear randomly in a virtual environment, find and focus virtual balls that appear randomly and pick up virtual spheres and transfer	VR: 2 h a wk for 5 wks	At the end of the 5-wk period: laparoscopic pig model (cameras and instrument navigation, moving 3 pieces of linen from one structure to another, transferring 3 pieces of plastic strips, removal of a suture and transfer, removal of clips and place them on the gallbladder)
Lucas *et al.*[115]	32 medical students	VR vs no training	VR: camera manipulation, hand-eye coordination, clipping, grasping and clipping, 2-handed maneuvers, cutting, fulguration, object translocation, followed by simulated procedures	6 sessions of 30 min	After training the 6 sessions: VR (CCE)
Lucas *et al.*[114]	32 medical students	VR vs no training	VR: camera manipulation, hand-eye coordination, clipping, grasping and clipping, two-handed maneuvers, cutting, fulguration, object translocation. This was followed by simulated procedures	6 sessions of 30 min	After training the 6 sessions: laparoscopic nephrectomy on pig model

Study	Participants	Interventions	Training tasks	Duration of training	Model used for assessment + task
Maschuw *et al.*[120]	50 PGY1 surgical residents	VR vs no training	VR: camera and instrument navigation, coordination, grasping, lifting and grasping, clip applying, diathermy-cutting	3 months	3 months after randomisation: VR (diathermy-cutting)
Seymour *et al.*[122]	16 PGY1-4	VR + standard laparoscopic training vs standard laparoscopic training	VR (difficult level): manipulate and diathermy until proficiency levels on 2 consecutive trials	1 h	Laparoscopic CCE on a patient
Tanoue *et al.*[122]	35 medical students	VR vs no training	VR: transfer place, tranversal, stretch diathermy, adjust needle, push needle, stitch, lift stitch, continuous suture, square knot and interrupted suture	2 h a day for 2 days	VT (one stitch and two ties between dots drawn on a rubber sheet)
Verdaasdonk *et al.*[124]	20 PGY 1-2 surgical residents	VR vs no training	VR: tying a surgical double knot	At least 10 times	Within a wk after training or no training: laparoscopic double knot tying on a pig
Ikehara *et al.*[127]	57	VR vs VT vs no training	Both training models: moving bead-like objects to target areas	n.r.	VR (grasping, lift + grasping and coordination)
Munz *et al.*[98]	24 medical students	VR vs VT vs no training	VT: instrument navigation, co-ordination, grasping; VR: same tasks	weekly sessions for 30 min for 3 wks	After 3 wks training: VT (cut out a circle and applying clips)

Study	Participants	Interventions	Training tasks	Duration of training	Model used for assessment + task
Pearson *et al.*[125]	43 students unable to tie an intracorporeal knot under 600 s	VR vs VT vs self-practice vs no training vs unstructured training	VR: grasping a sphere and placing in a cube, grasp sphere, transfer to other instrument and release in cube, tranversal; VT: slam dunk, cobra rope, terrible triangle; self-practice: knot tying in the trainer on a foam pad; unstructured: watching a video of three sutures being tied	VR: 10 repetitions of task 1-3 ; VT: 10 repetitions of task 1-3. Self practice: suturing on their own for 30 min	VT: 10 intracorporeal knots on a foam path
Tanoue *et al.*[123]	35 medical students	VR vs VT vs no training	VR: transfer place, tranversal, stretch and diathermy, suturing and knot tying; VT: pegboard pattern, running string, suturing, knot tying	2 h a day for 2 days	After training: VT (knot tying on a rubber sheet)
Torkington *et al.*[126127]	30 medical students	VR vs VT vs no training	VR: 6 training tasks. VT: placing chick peas on golf tees, passing matchsticks through hoops, cutting out shapes, stacking sugar cubes, unwrapping confectionery	VR: 10 repetitions of all tasks which were performed twice over a period of 1 h; VT: 1 h	VT: grasping and cutting sutures task
Van Sickle *et al.*[140]	24 PGY3,5, or 6 surgical residents	VT+VR vs standard training group	VR: tranversal till proficiency followed by training using the VT; VT: suturing and knot tying till proficiency using foam models with rubber tubing; Standard training group: access to VR and VT, but without formal training	5 wks for PGY3, 8 wks for PGY5 for 4 or 5 1 h sessions; VR: training till proficiency on 2 consecutive trials; VT: suturing in less than 60 s, fewer than 10 needle manipulations in 2 consecutive trials. Knot tying: performance quality score > 25 and knot tied in less than 60 s in 2 consecutive trials	Laparoscopic Nissen on a patient (2 consecutive knots)

Study	Participants	Interventions	Training tasks	Duration of training	Model used for assessment + task
Madan *et al.*[100]	65 medical students	VR+VT vs VR vs VT vs no training	VR: acquire and place, transfer and place, tranversal, withdraw and insert, diathermy, manipulate and diathermy; VT: placing pegs on a board, transferring pegs from one hand to another and then to the board, starting first with the dominant hand or non-dominant hand, placing a pipe cleaner through a tube, placing a probe through three rings, progressing from one end of the rope to the other; VR+VT: both training tasks	10 times 20 min. If training on both trainers: 10 min on the VR and 10 min on the VT during each session	Laparoscopic pig model (placing a piece of bowel in a bag, liver biopsy, placing a stapler on the bowel, running the bowel)
Korets et al.[149]	19 PGY1-5 urology residents	VR vs robot vs no training	VR: 15 exercises to train endowrist manipulation, camera movement and clutching skills, needle control; robot: endowrist manipulation, camera movement and clutching skills, knot tying	Robot: 90-min session	Robot: movement of rings through a wire and surgeon's knot followed by 2 knots for 2 additional times
Banks *et al.*[128]	20 PGY1 gynecologic residents	VT+apprenticeship teaching in the OR vs apprenticeship teaching	VT: suturing, knot tying, bilateral tubal ligation	2 h	Laparoscopic bilateral tubal ligation on a patient
Bruynzeel *et al.*[129]	30 medical students	VT versus mirror trainer versus no training	VT : knot-tying	VT : 6 knots during 3 consecutive days	next day : laparoscopic porcine model (closure of a colon perforation)

Study	Participants	Interventions	Training tasks	Duration of training	Model used for assessment + task
Clevin *et al.*[130]	16 gynecologic residents	VT vs no training	VT: moving pegs, cutting out a drawn circle, introducing a catheter into a tube, applying clips, cutting out the inner balloon of two balloons	VT: 3 h	After 1 wk: VR (camera navigation, instrument navigation, co-ordination, grasping, lifting and grasping, cutting, clip applying)
Coleman *et al.*[131]	26 PGY3-4 gynecologic residents	VT vs no training	VT: running string, block move, checkerboard drill, bean drop, suture foam	VT: at least 30 min daily for 10 days	Wk 4: VT (running string, block move, checkerboard drill, bean drop, suture foam) + laparoscopic partial salpingectomy on a patient
Derossis *et al.*[14]	12 PGY3 surgical residents	VT vs no training	VT: pegboard patterns, pattern cutting, clip application, placement of ligating loop, mesh placement over a defect, intracorporeal and extracorporeal knots	VT: 5 weekly sessions	7 wks after training: VT (pegboard patterns, pattern cutting, clip application, placement of ligating loop, mesh placement over a defect, intracorporeal and extracorporeal knots)
Fried *et al.*[138]	12 PGY3 surgical residents	VT vs no training	VT: transferring, cutting, clipping, ligating loop, mesh placement, intracorporeal knot, extracorporeal knot	VT: 5 weekly sessions	5 wks after training: VT and laparoscopic pig model (transferring, cutting, clipping, ligating loop, mesh placement, intracorporeal knot, extracorporeal knot)
Harold *et al.*[132]	17 junior and senior surgical residents	VT vs no training	VT: proctoring	VT: 60 min	VT (2 stitches)
Korndorffer *et al.*[133]	17 PGY1-5 surgical residents	VT vs no training	VT: suturing and intracorporeal knot tying	VT: 8 wks during 1-h weekly sessions till proficiency on 2 consecutive attempts	After 8 wks of training: laparoscopic pig model (3 gastrogastric sutures)

Study	Participants	Interventions	Training tasks	Duration of training	Model used for assessment + task
Scott *et al.*[134]	27 PGY2-3 surgical residents	VT vs no training	VT: checkerboard, bean drop , running string, block move and suture foam	VT: 30 min a day for 10 days	After at least 5 wks: VT (checkerboard, bean drop , running string, block move and suture foam - 3 times each task); laparoscopic CCE on a patient
Sroka *et al.*[135]	19 PGY1-3 surgical residents	VT + regular residency training vs regular residency training	VT: peg transfer, circle cut, placement of a ligating loop, simple suture tied with extra- and intracorporeal techniques until proficiency level	Training during free time	After a mean time of 145 days: VT (peg transfer, circle cut, placement of a ligating loop, simple suture tied with extra- and intracorporeal techniques); laparoscopic CCE on a patient
Stefanidis *et al.*[136]	18 medical students	VT vs no training	VT: laparoscopic suturing till proficiency level was achieved on 2 consecutive and 5 additional attempts	After 1 and 3 months	At 6 months: VT (laparoscopic suturing)
Stefanidis *et al.*[139]	32 medical students	VT vs VT (increased difficulty) vs no training	VT: laparoscopic suturing till proficiency level on 2 consecutive plus 10 additional attempts; VT (increased difficulty): the same, but in a constrained space, listening to OR noise, with a shorter suture and starting with a dropped needle	Once or twice a wk, maximally for 1 h	Laparoscopic pig model (gastrogastric suturing)
Traxer *et al.*[137]	12 PGY3-5 urology residents	VT vs no training	VT: checkerboard, bean drop, running string, block move and suture foam	30 min daily for 10 days	At 2 wks : laparoscopic nephrectomy on pig model

Study	Participants	Interventions	Training tasks	Duration of training	Model used for assessment + task
Madan *et al.*[151]	32 medical students	VT vs VT+VR	VT: placing pegs, transferring pegs, placing a pipe cleaner through a tube, placing a probe through different rings, progressing one end of the rope to the other; VR: acquire and place, transfer and place, tranversal, withdraw and insert, diathermy, manipulate and diathermy	VT: 10 sessions of 20 min; VT+VR: 10 sessions, 10 min VT and 10 min VR	No extra assessment tool; VT: comparison of the same 5 tasks during session 10
Chung *et al.*[157]	13 PGY2-5 surgical residents	VT+laparoscopic pig training vs VT	VT: make an "x" shape, transfer pieces from the "x" and place them on the poles, placement of 2 clips, thread the pipe cleaner into the rubber tube up to the black thread, pick up a green ball and pass it through three loops, suturing ; VT + laparoscopic porcine training : same task + Nissen and partial colectomy	VT: each task twice; laparoscopic pig training: 4h	After 2 days: VT (same 6 tasks)
Chmarra *et al.*[141]	19 gynaecology residents	VR vs VT	VR: ball task, ring task, elastic band task; VT: same tasks, but with force feedback	Each task one time on each trainer	Both models
Debes *et al.*[108]	46 medical students or interns	VR vs VT	VT : picking up and sorting pegs into 2 boxes, pick up and pass the pegs through an eyebolt and sort the pegs into 2 boxes, stacking 5 sticks on the back of a plastic donkey, running a 170-cm long silk ribbon, picking up and passing a pin through holes ; VR: core skills 1	8 sessions for all tasks	VT (difficult level): picking up a peg and passing it through eyebolts; VR: manipulate and diathermy

Study	Participants	Interventions	Training tasks	Duration of training	Model used for assessment + task
Diesen *et al.*[142]	23 medical students and surgical interns	VR vs VT	VR: camera skills, instrument handling, object positioning, dissection, ligation, suturing, knot tying. VT: same tasks	A minimum of 10 repetitions over a 6-month interval	2 and 6 months after starting the study: laparoscopic pig model (camera navigation, 2 eye-hand coordination, clipping and electrocautery and knot-tying within 5 minutes)
Hamilton *et al.*[107]	50 PGY1-2 surgical residents	VR vs VT	VR: grasping and transferring a sphere to a box, same task, but transfer of the sphere to the other grasper before transfer to the box, tranversing, replacing an instrument in the peritoneal cavity, cauterization of three targets, same task but use of the other hand; VT: suture foam, bean drop, triangle transfer, rope drill, checkerboard	VR: 10 one-half h sessions during 2 wks: 2 repetitions in all tasks, except task 3; VT: 10 one-half h sessions during 2 wks	During wk 4: subgroup PGY2 residents performed a laparoscopic CCE on a patient; total group: VR and VT: same tasks
Jordan *et al.*[143]	24 subjects without laparoscopic experience	VR vs VT (normal imaging)	VR: acquire and place, transfer and place, tranversal, withdraw and insert, diathermy, manipulate and diathermy; VT (alternating Y-axis and normal laparoscopy): same tasks; VT: 6 training trials on a laparoscopic cutting task	n.r.	VT: 2-minute cutting task
Kanumuri *et al.*[144]	16 medical students	VR vs VT	VR: interrupted suture task; VT: interrupted suture task	8 1 h sessions over the 4- wk rotation until proficiency. Subjects were allowed to train more	At the end of the 4-wk training: laparoscopic intracorporeal suturing and knot tying on a pig model

Study	Participants	Interventions	Training tasks	Duration of training	Model used for assessment + task
Kothari *et al.*[145]	29 medical students	VR vs VT	VR: acquire and place, transfer and place, tranversal, withdraw and insert, diathermy, manipulate and diathermy; VT: rope pass, cup drop, triangle transfer	VR: 5 times each task for 5 days; VT: each drill 5 times for 5 days	After 5 days: VT(mean time to perform 5 interrupted knots)
Loukas et al.[146]	44 medical students	VR vs VT	VR and VT: peg transfer, cutting, knot tying	Each task: 12 times, 2 sessions a day	Same task on the alternative simulator
McDougall *et al.*[147]	20 medical students	VR vs VT	VR: knot-tying, suturing; VT: same tasks	VR: a least 2 h of training or till proficiency; VT: at least 2 h of training	Within 1 wk of the training session and after 6 wks: laparoscopic suturing and knot tying of a 2 cm
Larsen *et al.*[101]	24 PGY1-2 gynecologic residents	VR vs standard laparoscopic training	VR: lifting and grasping, cutting, right salpingectomy; standard laparoscopic training: only simple laparoscopy or assisting laparoscopy	VR: once in each training cycle of 45-60 min, the salpingectomy was repeated during the remainder of the cycle. Training till proficiency in 2 consecutive simulations	After maximum 60 days: laparoscopic right salpingectomy on a patient
McClusky *et al.*[112]	12 PGY1-2 surgical residents	VR vs standard laparoscopic training	VR: diathermy task till proficiency	n.r.	Laparoscopic CCE on a patient
Zendejas et al.[102]	50 surgery residents (performed at least 1 TEP)	VR vs standard laparoscopic training	VR: TEP+regular clinical practice; Standard laparoscopic training: regular clinical practice	VR: till proficiency level (repair of both hernias in less than 2 min on 2 consecutive attempts)	Laparoscopic TEP on a patient
Halvorsen *et al.*[150]	26 medical students	VR vs robot	VR: placement of stitches to complete a virtual anastomosis; robot: suturing on a rubber glove with premarked dots	VR: 3 sessions of 40 min during 2 wks; Robot: 3 sessions of 40 min	After 2 wks of training: robot pig model (number of stitches placed between a vein graft and a coronary artery for 40 min)

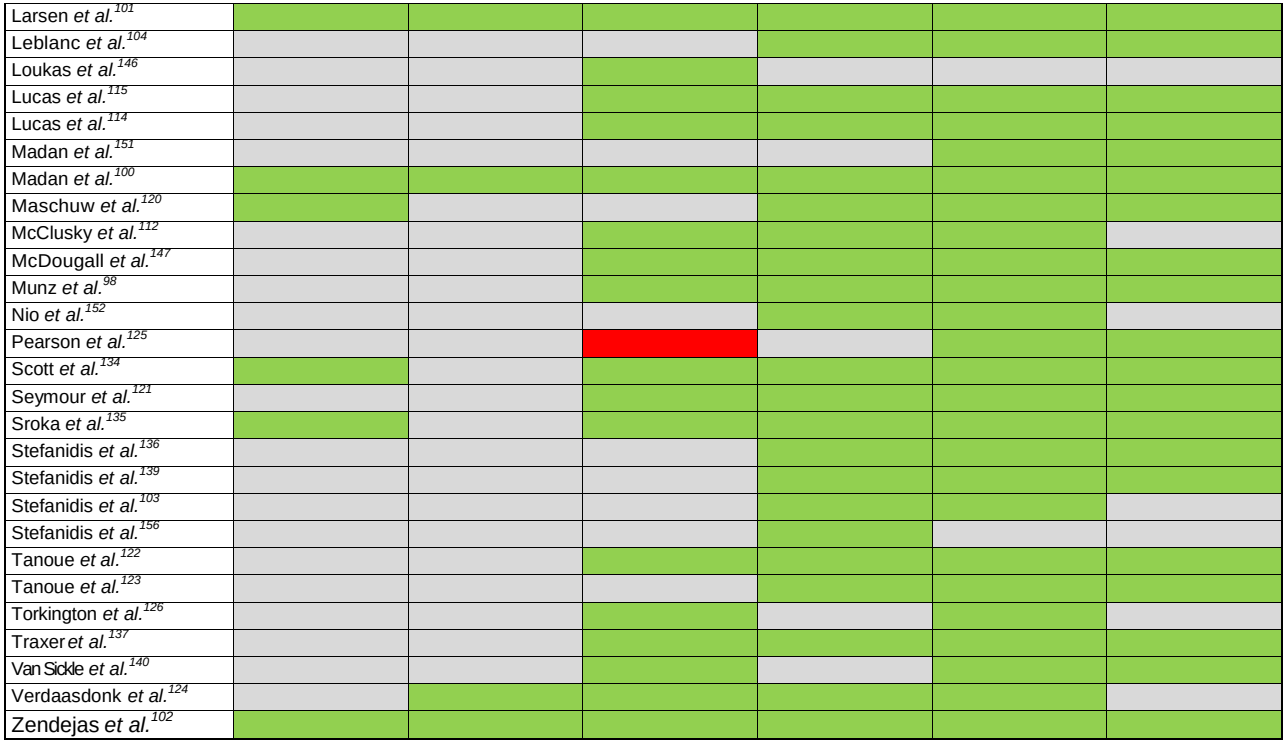
Study	Participants	Interventions	Training tasks	Duration of training	Model used for assessment + task
Katsavelis *et al.*[148]	8 medical students and research fellows	VR vs robot	Robot: a pick-and-place task, passing a surgical needle through 6 pairs of holes; VR: same tasks	5 trials of each task for each environment	Both models
Leblanc *et al.*[104]	34 trainers and trainees	VR vs laparoscopic human cadaver	Hand-assisted laparoscopic sigmoidectomy	One time performance of the procedure	no extra assessment tool
Blavier *et al.*[153]	40 medical students	VT (2D) vs VT (3D) vs robot (2D) vs robot (3D)	Passing a needle with a thread through rings	6 sessions: number of rings passed with the needle for 4 min	All 4 types of models
De Ugarte *et al.*[154]	12 PGY2 surgical residents	VT vs robot	Bead drop exercise, x-exercise, suturing, knot tying	3 sessions over a 3-wk period	Both models
Heemskerk *et al.*[155]	8 medical students	VT vs robot	Pick up and drop, give over the bead, cap the needle, suturing and knot tying	Every task was performed 3 times using each model; only suturing and knot tying was not performed on VT	Both models
Nio *et al.*[152]	20 medical students	VT vs robot	Dropping beads, rope-passing, needle-capping, suturing, laparoscopic CCE on a pig cadaver liver	Each task once	No extra assessment tool
Stefanidis *et al.*[156]	117 learning center attendees (51attendings, 32 fellows, 27	VT vs robot	both models: making a knot	Time limit was 5 min per task	Both models
Stefanidis *et al.*[103]	34 medical students	Laparoscopic pig training vs robot pig training	Both models: 3 gastrogastric sutures	Maximal allowed completion time was 10 min	Both models

CCE, cholecystectomy; n.r., not reported; PGY, post graduate year ; TEP, total extraperitoneal; VR, virtual reality; VT, video trainer

Appendix 2

Risk of bias summary

Study	Adequate sequence generation	Allocation concealment	Blinding of outcome assessors	Incomplete outcome data addressed	Free of selective reporting	Selection bias
Aggarwal *et al.*[99]						
Ahlberg *et al.*[117]						
Ahlberg *et al.*[118]						
Banks *et al.*[128]						
Blavier *et al.*[153]						
Bruynzeel *et al.*[129]						
Calatayud *et al.*[10]						
Chmarra *et al.*[141]						
Chung *et al.*[157]						
Clevin *et al.*[130]						
Coleman *et al.*[131]						
Debes *et al.*[108]						
Derossis *et al.*[14]						
De Ugarte *et al.*[154]						
Diesen *et al.*[142]						
Fried *et al.*[138]						
Grantcharov *et al.*[8]						
Halvorsen *et al.*[150]						
Hamilton *et al.*[107]						
Harold *et al.*[132]						
Heemskerk *et al.*[155]						
Hogle *et al.*[113]						
Hogle *et al.*[113]						
Hyltander *et al.*[119]						
Ikehara *et al.*[127]						
Jordan *et al.*[143]						
Kanumuri *et al.*[144]						
Katsavelis *et al.*[148]						
Korets *et al.*[149]						
Korndorffer *et al.*[133]						
Kothari *et al.*[145]						



Green for low risk of bias, red for high risk of bias, grey for unclear

Appendix 3

Data and analysis table

Training vs no training				
VR vs no training				
Author	*Time*	*Error score*	*Composite score*	*Economy of movement*
Aggarwal *et al.*[99]	4590 s (no) vs 2165 s (VR)		17 (no) vs 25 (VR)	Total path length: 169 m (no) vs 87 m (VR); total movements: 2446 (no) vs 1029 (VR)
Ahlberg *et al.*[117]			12.3±5.6 (no) vs 12.0±4.5 (VR) (mean±SD)	
Ahlberg *et al.*[118]		VR better		
Calatayud *et al.*[10]			28.5 (VR) vs 19.25 (no) (median)	
Grantcharov *et al.*[8]	VR better	VR better		VR better
Hogle *et al.*[113]		2.96±0.59 (VR) vs 3.10±0.53 (no) (mean±SD)		2.89±0.53 (VR) vs 2.82±0.62 (no)
Hogle *et al.*[113]		2.8±1.03 (VR) vs 2.55±0.52 (no) (mean ±SD)		2.7±0.82 (VR) vs 2.36±0.92 (no)

Training vs no training				
VR vs no training				
Author	*Time*	*Error score*	*Composite score*	*Economy of movement*
Hyltander *et al.*[119]	VR better for 5 out of 6 tasks		VR better	
Lucas *et al.*[115]	VR better: 3.22±1.22 (VR) vs 2.09±0.80 (no) (mean±SD)	VR better: 3.34±1.01 (VR) vs 2.13±0.79 (no) (mean±SD)	27.94±7.67 (VR) vs 17.25±6.16 (no)	
Lucas *et al.*[114]	2.5±1.2 (VR) vs 1.9±0.9 (no) (mean±SD)	3.1±1.2 (VR) vs 2.6±0.9 (VR) (mean±SD)	21.0±6.8 (VR) vs 15.7±6.6 (no) (mean±SD)	
Maschuw *et al.*[120]	141 s (VR) vs 241 s (no)	VR better: 2.28 (VR) vs 8.12 (no)		VR better: economy of instrument motion: 7.2 (VR) vs 22.16 (no); instrument path length 3.13 m (VR) vs 4.82 m (no)
Seymour *et al.*[121]	15 min (VR) vs 21 min (no)	VR better: 1.19 (VR) vs 7.38 (no)		
Tanoue et al.[123]	200.9 s (VR) vs 343.0 s (no)			
Verdaasdonk *et al.*[124]	262 (VR) vs 374 (no)	VR better: 24 (VR) vs 36 (no) (median)		

Training vs no training				
VR vs no training				
Author	*Time*	*Error score*	*Composite score*	*Economy of movement*
Ikehara *et al.*[127]	VR better			
Munz *et al*.[98]				
Pearson *et al.*[125]				
Tanoue *et al.*[123]	VR better			
Torkington *et al.*[126]				VT better
Madan *et al.*[100]	VR better			
VT vs no training				
Author	*Time*	*Error score*	*Composite score*	*Economy of movement*
Madan *et al.*[100]	VT better for 2 out of 4 tasks		VT better for 1 out of 4 tasks	
Munz et al.[98]		VT better		VT better
Tanoue *et al.*[123]	VT better			
Pearson *et al.*[125]				

Training vs no training				
VT vs no training				
Author	*Time*	*Error score*	*Composite score*	*Economy of movement*
Torkington *et al.*[126]				VT better
Ikehara *et al.*[127]	VT better			
Banks *et al.*[128]	VT better	VT better	VT better	
Bruynzeel *et al.*[129]	VT better			
Clevin *et al.*[130]	VT better	VT better		VT better
Coleman *et al.*[131]	222 s (VT) vs 329 s (no)		21.7 (VT) vs 20.3 (no)	
Derossis *et al.*[14]			1658±82 (VT) vs 1313±87 (no) (mean±SD)	
Harold *et al.*[132]	VT better: tie time difference: 599.9±231.4 s (VT) vs 323.9±254.0 s (no) (mean±SD)			
	tie time: 133.4±35.4 s (VT) vs 176.3±58.3 s (no) (mean±SD)			

Training vs no training				
VT vs no training				
Author	*Time*	*Error score*	*Composite score*	*Economy of movement*
Korndorffer *et al.*[133]	VT better		VT better	
Scott *et al.*[134]	VT better when tested on VT posttraining when tested on pigs	VT better: 0.3 (VT) vs 0.1 (no)	VT better: 0.7 (VT) vs 0.2 (0.2)	
Sroka *et al.*[135]		VT better: 1.13±1.0 (VT) vs 0.3±0.7 (no) (mean±SD)	VT better: 6.1±1.3 (VT) vs 1.8±2.1 (no) (mean±SD)	1.13±1.0 (VT) vs 0.4±1.1 (no) (mean±SD)
Stefanidis *et al.*[136]			VT better	
Stefanidis *et al.*[139]			VT better	
Traxer *et al.*[137]				
Fried *et al.*[138]			VT better: 248±53 % (VT) vs 135±15 % (no) (mean±SD)	

Training vs no training				
VT+VR vs no training				
Author	*Time*	*Error score*	*Composite score*	*Accuracy*
Madan *et al.*[100]	VT+VR better for 3 out of 4 tasks		VT better for 1 out of 4 taks	
Van Sickle *et al.*[140]	VT+VR better: 525.6±189.6 s (VT+VR) vs 789.5±171.3 s (no) (mean±SD)	VT+VR better: 25.6±9.3 (VT+VR) vs 37.1±10.2 (no) (mean±SD)		VT+VR better: 18.5±10.5 (VT+VR) vs 27.3±8.5 (no) (mean±SD)

VR vs other training forms				
VR vs VT				
Author	*Time*	*Error score*	*Composite score*	*Economy of movement*
Madan *et al.*[100]				
Hamilton *et al.*[107]	VT better (assessed on VT): 43.4±3.4 (VR) vs 64.3±2.9 (VT) (mean±SD)		VR better (assessed on VR): 55.5±1.2 (VR) vs 41.8±0.90 (VT) (mean±SD)	
Munz *et al.*[98]				
Debes *et al.*[108]	VR better (assessed on VR): 90.3 s (VR) vs 188.6 s (VT) assessed on VT: 402.0 s (VR) vs 325.6 s (VT)		VR better (assessed on VR): 224.7 (VR) vs 527.0 (VT)	VR better (assessed on VR): 4.40 (VR) vs 7.50 (VT) assessed on VT
Tanoue *et al.*[123]	VT better			
Pearson *et al.*[125]				
Torkington *et al.*[126]				
Ikehara *et al.*[127]				

VR vs other training forms				
VR vs VT				
Author	*Time*	*Error score*	*Composite score*	*Economy of movement*
Chmarra *et al.*[141]	VT-VR group better than VR-VT group (assessed on VT) for 1 out of 3 tasks			VT-VR group better than VR-VT groups (assessed on VT and VR) for 1 out of 3 tasks
Diesen *et al.*[142]				
Jordan *et al.*[143]		VR better		
Kanumuri *et al.*[144]	206 s (VR) vs 156 s (VT)		14 (VR) vs 17 (VT)	
Kothari *et al.*[145]	30±21 % (VT) vs 39±21 % (VR)			
Loukas *et al.*[146]	cross-over: VT and VR individually better for 2 out of 3 tasks			VR better
McDougall *et al.*[147]	40±15 min (VT) vs 41±10 min (VR) (mean±SD)			

VR vs other training forms				
VR vs standard laparoscopic training in the OR				
Author	*Time*	*Error score*	*Composite score*	*Economy of movement*
Larsen *et al.*[101]	VR better: 12 min (VR) vs 24 min (OR)		VR better: 33 (VR) vs 23 (OR)	
Zendejas *et al.*[102]	VR better	VR better	VR better	
McCLusky *et al.*[112]	VR better: 31 min (VR) vs 39 min (OR)	VR better: 11.7 (VR) vs 19.7 (OR)		
VR vs robot training				
Author	*Time*	*Error score*	*Composite score*	*Economy of movement*
Katsavelis *et al.*[148]	VR better for 1 out of 2 tasks			VR better for 1 out of 2 tasks
Korets *et al.*[149]				
Halvorsen *et al.*[150]				

VR vs other training forms				
VR vs cadaver training				
Author	*Time*	*Error score*	*Composite score*	*Economy of movement*
Leblanc *et al.*[104]			cadaver better for 1 out of 4 scores: trainers : 1.0±0.1 (cadaver) vs 1.1±0.3 (VR); trainees : 1.0±0.1 (cadaver) vs 1.1±0.2 (VR) (mean±SD)	
VT+VR vs VT training				
Author	*Time*	*Error score*	*Composite score*	*Economy of movement*
Madan *et al.*[151]				
VT vs robot training				
Author	*Time*	*Error score*	*Composite score*	*Accuracy*
Nio *et al.*[152]	VT better for 2 out of 5 tasks	Robot better for 1 out of 5 tasks		
Blavier *et al.*[153]		Robot better	Robot better	
De Ugarte *et al.*[154]	VT better for 3 out of 4 tasks			
Heemskerk *et al.*[155]	Robot better for 3 out of 5 tasks			Robot better for 2 out of 4 tasks
Stefanidis *et al.*[156]			VT better: 84±75 (VT) vs 56 ± 63 (robot)	

Laparoscopic animal training vs other training forms				
Laparoscopic animal training vs robot animal training				
Author	*Time*	*Error score*	*Composite score*	*Economy of movement*
Stefanidis et al.[103]	Robot better: 600 s (animal) vs 460 s (robot)	Robot better: 1 (animal) vs 0 (robot)	Robot better	
Laparoscopic animal training + VT vs VT				
Author	*Time*	*Error score*	*Composite score*	*Economy of movement*
Chung et al.[157]	Combined training better: 34.3± 5.7 (combined) vs 7.3±9.2 (VT)			

Green is statistically significant difference; Yellow is no significant difference; Grey is not evaluated. CCE, cholecystectomy; CI: confidence interval; SD, standard deviation; VR, virtual reality; VT, video trainer

Appendix 4

Search strategy MEDLINE through PubMed

#1 Postmortem or post-mortem and reperfusion or perfusion [title/abstract]
#2 #1 and cadaver* [title/abstract]
#3 #1 and corpse* [title/abstract]
#4 #1 and bod* [title/abstract]
#5 #1 and embalm* [title/abstract]
#6 #1 and fresh [title/abstract]
#7 #1 and thiel [title/abstract]
#8 #1 and formalin [title/abstract]
#9 #1 and paraffin* [title/abstract]
#10 #1 and peg [title/abstract]
#11 #1 and surg* [title/abstract]
#12 #1 and simulat* [title/abstract]
#13 #1 and teaching [title/abstract]
#14 #1 and education* [title/abstract]
#15 #1 and infusion model [title/abstract]
#16 #1 and research [title/abstract]
#17 #1 and experimental model [title/abstract]
#18 #1 and anatomy [title/abstract]
#19 #1 and angiography [title/abstract]
#20 #1 and endoscop* [title/abstract]
#21 #1 and laparoscop* [title/abstract]
#22 #1 and dissection [title/abstract]
#23 Postmortem or post-mortem and circulation [title/abstract]
#24 #23 and cadaver* [title/abstract]
#25 #23 and corpse* [title/abstract]
#26 #23 and bod* [title/abstract]
#27 #23 and embalm* [title/abstract]
#28 #23 and fresh [title/abstract]
#29 #23 and thiel [title/abstract]
#30 #23 and formalin [title/abstract]
#31 #23 and paraffin* [title/abstract]
#32 #23 and peg [title/abstract]
#33 #23 and surg* [title/abstract]
#34 #23 and simulat* [title/abstract]
#35 #23 and teaching [title/abstract]
#36 #23 and education* [title/abstract]
#37 #23 and infusion model [title/abstract]
#38 #23 and research [title/abstract]
#39 #23 and experimental model [title/abstract]
#40 #23 and anatomy [title/abstract]
#41 #23 and angiography [title/abstract]
#42 #23 and endoscop* [title/abstract]
#43 #23 and laparoscop* [title/abstract]
#44 #23 and dissection [title/abstract]
#45 Cadaver* [title/abstract]
#46 #45 and *perfusion [title/abstract]
#47 #45 and infusion model [title/abstract]
#48 #45 and extracorporeal circulation [title/abstract]
#49 #45 and experimental model [title/abstract]
#50 #45 and pump* [title/abstract]
#51 #45 and pulsatile[title/abstract]
#52 #45 and pressur* [title/abstract]
#53 #45 and surgical training [title/abstract]
#54 #45 and revascular iz* [title/abstract]
#55 #45 and reperfusion not animal [title/abstract]
#56 #45 and simulation [title/abstract]
#57 #45 and surg* [title/abstract]
#58 #45 and live surg* [title/abstract]
#59 #43 surgical technique [title/abstract]
#60 #45 and laparoscop* [title/abstract]
#61 #45 and microsurgery [title/abstract]
#62 #45 and minimally invasive [title/abstract]
#63 #45 and imag* [title/abstract]

#64 #43and dissect* [title/abstract]
#65 #45 and circulat* [title/abstract]
#66 #45 and bleeding [title/abstract]
#67 #45 and blood flow [title/abstract]
#68 #45 and perfus* [title/abstract]
#69 #45 and thiel [title/abstract]
#70 #45 and formalin [title/abstract]
#71 #45 and paraffin* [title/abstract]
#72 #45 and peg [title/abstract]
#73 #45 and teaching [title/abstract]
#74 #45 and education [title/abstract]
#75 Bod* [title/abstract]
#76 #75 and *perfusion [title/abstract]
#77 #75 and infusion model [title/abstract]
#78 #75 and extracorporeal circulation [title/abstract]
#79 #75 and experimental model [title/abstract]
#80 #75 and pump* [title/abstract]
#81 #75 and pulsatile[title/abstract]
#82 #75 and pressur* [title/abstract]
#83 #75 and surgical training [title/abstract]
#84 #75 and revascular iz* [title/abstract]
#85 #75 and reperfusion not animal [title/abstract]
#86 #75 and simulation [title/abstract]
#87 #75 and surg* [title/abstract]
#88 #75 and live surg* [title/abstract]
#89 #73 and surgical technique [title/abstract]
#90 #75 and laparoscop* [title/abstract]
#91 #75 and microsurgery [title/abstract]
#92 #75 and minimally invasive [title/abstract]
#93 #75 and imag* [title/abstract]
#94 #73and dissect* [title/abstract]
#95 #75 and circulat* [title/abstract]
#96 #75 and bleeding [title/abstract]
#97 #75 and blood flow [title/abstract]
#98 #75 and perfus* [title/abstract]
#99 #75 and thiel [title/abstract]
#100#75 and formalin [title/abstract]
#101#75 and paraffin [title/abstract]
#102#75 and peg [title/abstract]
#103#75 and teaching [title/abstract]
#104#75 and education [title/abstract]
#105Corpse* [title/abstract]
#106#105 and *perfusion [title/abstract]
#107#105 and infusion model [title/abstract]
#108#105 and extracorporeal circulation [title/abstract]
#109#105 and experimental model [title/abstract]
#110#105 and pump* [title/abstract]
#111#105 and pulsatile[title/abstract]
#112#105 and pressur* [title/abstract]
#113#105 and surgical training [title/abstract]
#114#105 and revascular iz* [title/abstract]
#115#105 and reperfusion not animal [title/abstract]
#116#105 and simulation [title/abstract]
#117#105 and surg* [title/abstract]
#118#105 and live surg* [title/abstract]
#119#103 surgical technique [title/abstract]
#120#105 and laparoscop* [title/abstract]
#121#105 and microsurgery [title/abstract]
#122#105 and minimally invasive [title/abstract]
#123#105 and imag* [title/abstract]
#124#103and dissect* [title/abstract]
#125#105 and circulat* [title/abstract]
#126#105 and bleeding [title/abstract]

#127#105 and blood flow [title/abstract]
#128#105 and perfus* [title/abstract]
#129#105 and thiel [title/abstract]
#130#105 and formalin [title/abstract]
#131#105 and paraffin [title/abstract]
#132#105 and peg [title/abstract]
#133#105 and teaching [title/abstract]
#134#105 and education [title/abstract]

Appendix 5

Characteristics of vascular reperfusion techniques

Study	Reperfusion system	Perfused vessels	Cannulated vessels	Vascular flow (l/min) vs pressurized vessels	Intravascular pressure (mmHg)	Arterial pulsations (ppm)	Type of perfusate
Aboud *et al*.[170]	IABP	A and V	Bilateral CA, VA and JV	A and V: pressurized	A: 80, V: 20–40	60	Tap water; A: light red, V: dark red
Aboud *et al.*[183]	Pulsatile pump	A and V	n.r.	A and V: pressurized	n.r.	Present	Tap water; A: light red, V: dark red
Aboud *et al*.[96]	Pulsatile pump	A and V	CA, FA, JV and FV	A and V: pressurized	A: 120/80, V: 15	100	Tap water; A: light red, V: dark red
Aboud *et al.* [95]	IABP	A and V	CA, FA, JV and FV	A and V: flow	A: 80–150, V: 15	120	Tap water; A: light red, V: dark red
Arbatli *et al.*[176]	Roller pump	A	Bilateral CA, one subclavian A, ascending and descending thoracic Ao	A: flow	A: 70	Present	Transparent saline solution (37°C)

Study	Reperfusion system	Perfused vessels	Cannulated vessels	Vascular flow (l/min) vs pressurized vessels	Intravascular pressure (mmHg)	Arterial pulsations (ppm)	Type of perfusate
Bouma *et al.*[28]	IABP	n.a.	Left ventricle	A: pressurized	n.r.	60-80	Saline solution and blood
Canaud *et al.*[180]	Pump	A	Proximal and distal Ao	A: flow: 10	A: 300/150	60	Transparent glycerin (30 %) and water (70 %)
Canaud *et al.*[181]	Pump	A	Proximal and distal Ao	A: flow: 10	A: 150/80	60	Transparent glycerin (30 %) and water (70 %)
Carey *et al.*[94]	Pump	A and V	FA, FV; separated filling of extremities and neck	A and V: pressurized	A: 120/90	Absent	Tap water, corn syrup and sodium chloride; A: red, V: blue
Chapter 11 of this thesis	Modified heart-lung machine	A	FA and FV	A: pressurized	n.a.	Present	Transparent PL (50°C)
Faure *et al.*[182]	Pulsatile pump	A	Proximal and distal Ao	A: flow: 10	A: 120/80	60	Transparent glycerin (30 %) and water (70 %)

Study	Reperfusion system	Perfused vessels	Cannulated vessels	Vascular flow (l/min) vs. pressurized vessels	Intravascular pressure (mmHg)	Arterial pulsations (ppm)	Type of perfusate
Garrett[97]	Pump	A	Bilateral CA, axillary, iliac, dorsalis pedis and posterior tibial A	A: flow	n.a.	Feasible	Red crystalloid solution
Gragnaniello *et al.*[171]	Intra-aortic balloon pump	A	CA and VA	A: pressurized	A: 100 (group 2)	Absent	Group 1: red silicone rubber; Group 2: red water and human serum
Güvencer *et al.*[172]	Pump	A and V	CA, VA and internal JV	A and V: flow	A: 80, V: 40	60	Tap water (22–24°C); A: red, V: blue
Jongkind *et al.*[177]	Roller pump	A	Left ventricle, ascending Ao and Ao branches at remote sites	A: flow	A: 120/80	Present	Red crystalloid solution
Kawashima *et al.*[168]	n.r.	A and V	Cerebral circle; vessels n.r.	A: pressurized	n.r.	Absent	Coloured silicone

Study	Reperfusion system	Perfused vessels	Cannulated vessels	Vascular flow (l/min) vs. pressurized vessels	Intravascular pressure (mmHg)	Arterial pulsations (ppm)	Type of perfusate
Kawashima *et al.*[169]	n.r.	A and V	Cerebral circle; vessels n.r.	A: pressurized	n.r.	Absent	Coloured silicone
Linsen *et al.*[178]	Pump	A	Descending Ao and bilateral FA	A: flow	n.r.	Present	Red crystalloid solution
Malikov *et al.*[30]	Manual injection	A	Axillary A	A: pressurized	A: 120	Absent	Rhodorsil
Numan *et al.*[179]	Roller pump	A	Proximal ascending and supraceliac Ao	A: flow	A: 70	Absent	Heated transparent saline solution
Olabe *et al.*[175]	Drip regulator	A	CA and VA	A: pressurized	A: 110	Present	Transparent tap water
Olabe *et al.*[173]	Drip regulator	A	CA and VA	A: pressurized	A: 110	Absent	Transparent tap water
Pham *et al.*[92]	Pump	A	FA	A: pressurized	A: 130/80	n.r.	Red saline solution

Study	Reperfusion system	Perfused vessels	Cannulated vessels	Vascular flow (l/min) vs. pressurized vessels	Intravascular pressure (mmHg)	Arterial pulsations (ppm)	Type of perfusate
Russin *et al.*[93]	Centrifugal pump	A and V	FA and FV	A and V: pressurized	A: 750, V: n.r.	Absent	Tap water (9.46 L), red food dye (10 mL) and saline solution (237 mL)
Scaglioni *et al.*[29]	Manual injection	A	External CA	A: pressurized	n.a.	Absent	Latex and barium sulfate
van Doormaal *et al.*[174]	IABP	A	CA and VA	A: pressurized	A: 110/80	Present	Red tap water
Wolff *et al.*[59]	Pump	A and V	Brachial, peroneal, deep circumflex iliac, inferior epigastric A and descending branch of circumflex FA	A and V: flow	A: 120/80	35	Red tap water

A, artery; Ao, aorta; CA, carotid artery; FA, femoral artery; FV, femoral vein; IABP, intra-aortic balloon pump; JV, jugular vein; mmHg, millimeter mercury; n.a., not applicable; n.r., not reported; ppm, pulsations per minute; PL, Paraffinum Liquidum; V, vein; VA, vertebral artery

Appendix 6

Advantages and limitations of reperfused human cadaver models

Study	Advantages	Limitations	Workshop offered regularly
Aboud *et al.*[170]	High fidelity, inexpensive, readily available	Partially fixed specimens do not last long, formalin bodies are stiff, no haemostasis	No
Aboud *et al.*[183]	Various techniques can be practised, substitute for anesthetised animals	n.r.	No
Aboud *et al.*[96]	Good for surgical training, inexpensive, readily available	n.r.	No
Aboud *et al.*[95]	Various techniques can be practised, inexpensive, good simulation of trauma scenarios, long-lasting cadavers	Fresh cadavers useful for 2 wks, formalin bodies are stiff and smell, tissues differ from patients, one-third of participants found training different from life scenarios, no blood-like perfusate	Yes
Arbatli *et al.*[176]	Flexibility and conditions similar to lifelike situation, minimal edema due to limited circulation time	Only one conduct implanted because of technical problems and anatomical limitations	No
Bouma *et al.*[28]	High fidelity and effective OPCAB model, easily reproducible, long lasting and repeated use due to Thiel embalming	High cost (€3 765/training day), limited availability of cadavers	Yes

Study	Advantages	Limitations	Workshop offered regularly
Canaud *et al.*[180]	Good model to assess feasibility and proximal angulation of stent grafts	Non-aneurysmal aortas, positioning of graft differs from *in vivo* situation	No
Canaud *et al.*[181]	*In situ* retrograde fenestration is feasible and reproducible, accurate placement of fenestrations, less reliance on preoperative imaging, non-customised stent grafts reduce cost and broaden availability, no side branch catheterisation needed	Non-aneurysmal aortas, preparation of specimens	No
Carey *et al.*[94]	Safe and high-fidelity model for teaching plastic surgery, good assessment of arterial flow using ICG	Health risks associated with fresh cadaver use, long model preparation time, high cost, edema, no haemostasis, inadequate venous outflow, no venous anastomosis feasible, quality of arterial anastomosis cannot be assessed	Yes
Chapter 11 of this thesis	Filled vessels allow transfer of valves, handling and tracking of catheters; radiographic control of implanted valves	Difficult to control contrast injection due to high viscosity and retrograde flow	No
Faure *et al.*[182]	Angioscopy and training in endovascular treatment of type B aortic dissection are feasible	Healthy aortas, dissection not always established until distal aorta, no haemostasis	No

Study	Advantages	Limitations	Workshop offered regularly
Garrett[97]	Almost lifelike model to perform all types of endovascular procedures with different sheaths, wires and catheters; radiographic assessment possible	Impossible to retrieve stent, edema	Yes
Gragnaniello *et al.*[171]	Tumor model	n.r.	Yes
Güvencer *et al.*[172]	Various techniques and haemostasis can be practised, good simulation of vascular trauma, prolonged tissue preservation	Tissues are stiff, no haemostasis	No
Jongkind *et al*[177]	Use of shorter delivery devices with better control, which may reduce operation time; creation of artificial aneurysms	Stiff formalin-fixed lung must be removed, procedure impossible in presence of excessive arterial calcification, advanced thoracoscopic and endovascular skills required	No
Kawashima *et al.*[168]	n.r.	n.r.	No
Kawashima *et al.*[169]	n.r.	n.r.	No
Linsen *et al.*[178]	Lifelike model	Fenestrations prone to infolding due to required endograft oversizing	No
Malikov *et al.*[30]	Long arterial pedicle can be dissected, good flap malleability, adequate covering of irregular defects	Flap not useful in distal wounds, no vascular anastomosis performed	No

Study	Advantages	Limitations	Workshop offered regularly
Numan *et al.*[179]	Few surgical and endoscopic skills required, technique can prevent embolic showers and facilitate achievement of cerebral hypothermia	Fixation of proximal stent end not secure	No
Olabe et al.[175]	Simple and inexpensive model	Limited availability of organs, edema	No
Olabe *et al.*[173]	Lifelike model	edema, no haemostasis	No
Pham *et al.*[92]	Lifelike model, training also possible on body parts, easily reproducible	No haemostasis	Yes
Russin *et al.*[93]	Lifelike model, pressurisation allows testing of anastomosis	Long and skilled model preparation, difficulty of obtaining cadavers, lack of pulsation, no haemostasis	No
Scaglioni *et al.*[29]	Versatile perforator flap based on superficial temporal artery, acceptable cosmetics	n.r.	No

Study	Advantages	Limitations	Workshop offered regularly
van Doormaal *et al.*[174]	Easy installation of the model, SELANA technique is feasible but needs improvement	One surgeon performed procedures, no haemostasis, different tissue feeling and decreased flap retrieval rate due to formalin fixation	No
Wolff *et al.*[59]	No edema, prolonged usage, easy identification of reperfused vessels, pulsations and skin staining	Thiel stiffer and less optimal than fresh cadavers, venous return weak and not constant, tap water leaks	Yes

ICG, indocyanine green; n.r., not reported; OPCAB, off-pump coronary artery bypass; SELANA, suture-less excimer laser-assisted non-occlusive anastomosis

Acknowledgments

Without doubt my first thanks go to Professor Piet Pattyn, my promotor. At the end of my surgical training, I was looking for an alternative non-obvious surgical career in combination with research. Professor, thank you for giving me the opportunity to conduct this ambitious and innovative project under your supervision. From the beginning my position was uncertain and many colleagues were skeptical concerning its feasibility, but you strongly believed in it. During these years, you were always available to guide me in the right direction when needed. You remained positive and supportive. Today, I already made a huge step towards complete human body reperfusion. The work is not finished yet, but I know that this model will further interest you. Again, many thanks for this unique chance. I won't forget.

My gratitude goes out to Professor Katharina D'Herde, my co-promotor. Thanks a lot for giving me the opportunity to reperfuse Thiel cadavers. Your contribution and critical revisions of the manuscripts guided me through the process of scientific publication. You organized meetings with experts, which gave insight into vascular reperfusion and reorientated my experiments. You also gave me the chance to present my findings on anatomical congresses. I hope I can work together with your team in the future because many interesting research projects in this field can be done.

Professor Filip De Somer, you have contributed enormously and effortlessly to the startup of the initial experiments. I know you will be further involved in this reperfused model. Thank you very much.

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Professor Wim Ceelen, head of the lab of experimental surgery, dear colleague, scientist and friend. To me, your surgical and scientific career is very inspiring. During the past years you gradually introduced me into writing scientific publications; oncological research; debulking and HIPEC surgery; and innovative cancer therapies like E-PIPAC. Many thanks.

Professor Yves Van Nieuwenhove, staff member at the department of gastrointestinal surgery. At the end of my surgical training we often talked about my future in surgery. You convinced me that an academic surgical career has many fascinating aspects. You were always interested in my experiments and helped me to focus on answering the hypothesis of this thesis. Thanks a lot.

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Dr. Ingrid Van Overbeke, veterinarian in the animalarium. Thanks for your support and professional approach during the pig experiments. Deborah Croes, animal care worker in the lab. Surely, Mieke learned you all the necessary things to run the cardiovascular lab. I hope we further have a good professional collaboration.

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Caroline, my love and greatest support. Thank you for listening, for helping me to carry-on and for taking care of Marie and Tijl when I was again absent during this very busy period in our lives. I appreciate you very much and truly adore you.

Marie, you do not understand yet why daddy is a medical doctor and operates on pigs and you were very eager to be on stage with me for my PhD defense. As promised, I will take some days off soon. Tijl, I am often your rival and when you do not find me at home you are always reassured that I am in the hospital. I will make more time for you now.

Dear family and in-laws, thank you for your interests in this research and your support! Mum and dad, thank you for raising me to be a loving and caring son, husband, father and friend. Pieter and Lotje, thank you for all the happy memories we share. I look back with pleasure on the period we lived together with mum and dad. Thanks to them we share a lot of interests. I wish you all the best to live your life as you intend. You are in my heart.

Curriculum Vitae

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Date of birth February 10th 1979

Place of birth Roeselare

Nationality Belgian

Civil status Married to Caroline Ducatelle

Children Marie, born August 3th 2009
Tijl, born June 30th 2012

Education

High school Sint-Aloysius College, Diksmuide
Main topics: Maths and Science
1997: diploma high school

Medical school Faculty of Medicine, Ghent University, Belgium
2004: Medical degree, cum laude

Training in surgery

General Surgery

2004-2008	Onze-Lieve Vrouw Hospital, Aalst, Belgium (Dr.J.P.Gillardin and Dr.H.Vanermen)
2008-2010	Ghent University Hospital (Prof.Dr.B.de Hemptinne)

Board certified in General Surgery 2010

Gastrointestinal Surgery

2010-2014	Clinical and Research fellowship at department of gastrointestinal surgery, Ghent University Hospital (Prof.Dr.P.Pattyn)

Research training

2011-2015	PhD student and member of doctoral schools of Life Sciences and Medicine, Ghent University

Current position

Since 10/2013	Staff member at department of gastrointestinal surgery, Ghent University Hospital (Prof.Dr.P.Pattyn)
Since 03/2014	Subspecialization in gastrointestinal oncology surgery: debulking and HIPEC; E-PIPAC (Prof.W.P.Ceelen)

Membership

2010	Royal Belgian Society of Surgery
2013-2014	European Society of Surgical Research
2013	European Society for Medical Oncology

Grants

2012-2016	Clinical research grant, Ghent University Hospital

Awards and prizes

30/11/2009	Winner of postgraduate surgery: casuistry: ‘Encrapment’, Ghent University Hospital

Achievements

03/2014	Reviewer Editorial Board of Frontiers in Surgical Oncology (Switzerland)
2014	Founding member of ITCIT (Instituut voor Training en Chirurgische Innovatieve Technieken), Ghent University Hospital
23/09/2015	Performed first E-PIPAC (Electrostatic Pressurized IntraPeritoneal Aerosol Chemotherapy) in BeNeLux at Ghent University Hospital
10/2015	Reviewer of Acta Chirurgica Belgica (Belgium)

Occasional reviewer of Clinical Anatomy (USA)

Certifications

2002	Electrocardiography, Ghent University
2004	Radioprotection, Ghent University
2011	Laboratory animal science I and II (Category C), Ghent University
2012	Advanced academic English writing skills, Ghent University
2013	Advanced academic English conference skills, Ghent University
2013	Good Clinical Practice, Istanbul, Turkey
2013	Methods in Clinical Cancer Research, Flims, Switzerland
2015	Doctoral schools of Life Sciences and Medicine, Ghent University

Training periods & courses abroad

09/2002	Student job at the department of Radiotherapy, Ghent University Hospital
07/2002	Electrocardiography, Ghent University
03-06/2004	Radioprotection, Ghent University
25-27/04/2007	Laparoscopic surgery, warm-up package, ircad/eits, Strasbourg, France
16-18/04/2008	Laparoscopic surgery, warm-up package, ircad/eits, Strasbourg, France
10/2008	Laparoscopic surgery, starters package, ircad/eits, Strasbourg, France
05/2009	Advanced suturing, Catharina Hospital, Eindhoven, The Netherlands
05/2009	Visiting surgeon, Imperial College London, St. Mary's Hospital, UK (A.Darzi)
21-23/10/2009	Laparoscopic surgery, starters package, ircad/eits, Strasbourg, France
04-14/10/2010	Laboratory animal science I+II, Ghent University
20-21/12/2010	Visiting surgeon, forensic center, CURML, Lausanne, Switzerland (S.Grabherr)
10/2011-12/2011	Advanced academic English writing skills, UCT, Ghent University
11/2011	Fundamentals of technology transfer, UCT, Ghent University
01-02/2012	Personal effectiveness, Ghent University

05/2012	Extra levator abdomino perineal excision of the rectum (ELAPE), Pelican Centre, Basingstoke, UK (B.Heald)
08/2012	Clinical studies: study design, implementation and reporting, Ghent University Hospital
02-05/2013	Advanced academic English conference skills, Ghent University
22-28/06/2013	15th ECCO-AACR-EORTC-ESMO Workshop on Methods in Clinical Cancer Research, Flims, Switzerland
18-19/10/2013	Current indications and techniques of flexible endoscopy for surgeons, ircad/eits, Strasbourg, France
19/09/2014	Visiting surgeon, NASCE (Network of Accredited Clinical Skills Centres in Europe) constitutional and council meeting, Stockholm, Sweden
18-19/05/2015	Workshop on Pressurized IntraPeritoneal Aerosol Chemotherapy (PIPAC), Universitätsklinikum der Ruhr-Universität, Marien Hospital, Bochum, Germany (M.A.Reymond)

Lectures as invited speaker

09/11/2006	Postgraduate surgery, casuistry: daling algemene toestand vanuit een onverwacht hoek na laparoscopische colonresectie, Ghent University Hospital
17/11/2008	Postgraduate surgery, casuistry: Hole-in-one, Ghent University Hospital
30/11/2009	Postgraduate surgery: casuistry: 'Encrapment', Ghent University Hospital
08/11/2011	Medical imaging in GI-surgery, LOK surgery, Het Pand, Ghent
29/03/2012	On-pump revascularization of the human corpse. Preliminary results. Presentation for Covidien employees, Het Pand, Ghent
05/02/2014	Reperfusion of Thiel embalmed tissue; PhD meeting of the department of Basic Medical Sciences, Ghent University Hospital
14/03/2014	Vasculaire reperfusie van Thiel gebalsmend weefsel. Wetenschapsdag Ghent University Hospital, Het Pand, Ghent (Presented by D.Van De Putte)
17/06/2014	Vascular reperfusion of Thiel embalmed tissues. Presentation for Covidien employees, Het Pand, Ghent
06/01/2015	Reperfusion of Thiel embalmed tissues. Presentation for NASCE (The Network of Accredited Clinical Skills Centres of Europe) visit, Ghent University Hospital
14/02/2015	Total mesocolic excision: is more better? Symposium: lymph nodes and cancer: insights for the surgical oncologist. Organised by the Belgian Society for Surgical Oncology, Het Pand, Ghent

Publications

A1 Articles in international journals included in the Science Citation index

M. Bakker, K. Van Vaerenbergh, W. De Gersem, M. Coghe, T. Boterberg, C. Derie, **W. Willaert**, W. Duthoy, L. Vakaet, W. De Neve Quality aspects and time gain of an automated procedure for generating an optimized plan in the routine treatment of breast cancer with

external tangential beam irradiation. EJC Supplements 09/2003; 1(5). DOI:10.1016/S1359-6349(03)90536-2**.** (Impact facor 9,39).

Van Vaerenbergh K, De Gersem W, Vakaet L, Coghe M, Boterberg T, Bakker M, Derie C, **Willaert W**, Seij P, Duthoy W, De Wagter C, De Neve W. Automatic generation of a Plan optimization volume for tangential field breast cancer radiation therapy. Strahlentherapie und Onkologie. 2005 Feb;181(2):82-8. (Impact factor 3.567).

W. Willaert, M. Petrovic, I. Van Herzeele, C. Randon, D. Voet, F. Vermassen. Treatment of iatrogenic femoral pseudoaneurysms by ultrasound percutaneous thrombin injection: effectiveness and complications. Acta Clinica Belgica 2006; 61: 19-23 (Impact factor 0,532).

Nesher N, Bakir I, Casselman F, Degrieck I, Degeest R, Wellens F, **Willaert W**, Vermeulen Y, Vanermen H, Van Praet F. Robotically enhanced minimally invasive direct coronary artery bypass surgery: a winning strategy? Journal of Cardiovascular Surgery (Torino). 2007 Jun; 48(3):333-8 (Impact factor 1.37).

Willaert W, Berrevoet F, De Bacquer D, Rogiers X, Troisi R. Open Preperitoneal Techniques versus Lichtenstein Repair for Inguinal Hernia. *Cochrane Database of Systematic Reviews* 2009, Issue 4. Art. No.: CD008034. DOI:10.1002/14651858.CD008034. Protocol (Impact factor 5.653).

W.Willaert, P.Pattyn, D.Van De Putte, K.Van Renterghem, Y.Van Nieuwenhove, W.Ceelen. New insights into the surgical anatomy of the rectum: a review. Acta Chirurgica Belgica. 2011 Sep-Oct; 111(5): 261-272 (Impact factor 0.432).

Willaert W, De Bacquer D, Rogiers X, Troisi R, Berrevoet F. Open Preperitoneal Techniques versus Lichtenstein Repair for Inguinal Hernia. *Cochrane Database of Systematic Reviews* 2012, Issue 7. In press Art. No.: CD008034. DOI: 10.1002/14651858.CD008034.pub2 (Impact factor 6.186).

W.Willaert, I Van Herzeele, W.Ceelen, D.Van De Putte, F.Vermassen, P.Pattyn. Endovascular Treatment of an Iatrogenic Perforation of the Internal Iliac Vein: a Case Report. Annals of vascular surgery 2012 Jul; 26(5): 733.e1-4. (Impact factor 1.332).

W. Willaert, Y. Van Nieuwenhove, T. Henckens, D. Van De Putte, K. Van Renterghem, W. Ceelen, P. Pattyn. Life-threatening side effects of malabsorptive procedures in obese patients necessitating conversion surgery: a review of 17 cases. Acta Chirurgica Belgica. 2012,112, 268-274 (Impact factor 0.432).

Wouter Willaert, Dirk Van De Putte, Katrien Van Renterghem, Yves Van Nieuwenhove, Wim Ceelen, Piet Pattyn. Training models in laparoscopy: a systematic review comparing their effectiveness in learning surgical skills. Acta Chirurgica Belgica. 2013, 113, 77-95 (Impact factor 0.44).

Wouter Willaert, Filip De Somer, Silke Grabherr, Katharina D'Herde, Piet Pattyn. Post-mortem reperfusion of a pig: a first step to a new surgical training model? Indian Journal of Surgery. 2013 Indian J Surg DOI 10.1007/s12262-013-0961-x (Impact factor 0.27)

Wouter Willaert, Dirk Van De Putte, Yves Van Nieuwenhove, Piet Pattyn, Wim Ceelen. Lymphatic spread, nodal count and the extent of lymphadenectomy in cancer of the colon. Cancer Treatment Reviews. 2013 Sep 25. pii: S0305-7372(13)00202-8. doi: 10.1016/j.ctrv.2013.09.013. (Impact factor 6.47).

Félix Gremonprez, **Wouter Willaert**, Wim Ceelen. Intraperitoneal chemotherapy (IPC) for peritoneal carcinomatosis: review of animal models. Journal of Surgical Oncology 2013 Oct 12. doi: 10.1002/jso.23464. (Impact factor 2.84).

Chevallier Christine, **Willaert Wouter**, Kawa Emilia, Centola Marcos, Steger Beat, Dirnhofer Richard, Mangin Patrice, Grabherr Silke. Post mortem circulation: An essential technique even for the living? Clinical Anatomy. 2013 Dec 21. doi: 10.1002/ca.22357 (Impact factor 1.16).

Wouter Willaert, Tom Van Hoof, Filip De Somer, Silke Grabherr, Katharina D'Herde, Wim Ceelen, Piet Pattyn. Postmortem revascularization of porcine lungs: a feasibility study. European surgical research. 2014, 52(1-2):8-20. doi: 10.1159/000357818. (Impact factor 1.43)

Wouter Willaert, Marie De Vos, Tom Van Hoof, Piet Pattyn, Katharina D'Herde. Understanding Thiel embalming in pig kidneys to develop a new circulation model. Plos One 2015 Mar 25;10(3):e0120114. doi: 10.1371/journal.pone.0120114. (Impact factor 3.53). Data available on Figshare: http://dx.doi.org/10.6084/m9.figshare.1297886.

Wouter Willaert, Wim Ceelen. The extent of surgery in cancer of the colon: is more better? World Journal of Gastroenterology. 2015; 21(1): 132-138. (Impact factor 2.43).

L.F. Abreu de Carvalho, V.scuderi, H.maes, P.Cupo, B.Geerts, M. Van Bockstal, F.Gremonprez, **W.Willaert**, P Pattyn, W.Ceelen, R.I.troisi. Simultaneous parenchymateous-preserving liver resection, cytoreductive surgery and intraperitoneal chemotherapy for stage IV colorectal cancer. Acta Chirurgica Belgica (impact factor 0.408).

Wouter Willaert, Francesca Tozzi, Tom Van Hoof, Piet Pattyn, Katharina D'herde. Investigation of Thiel embalming and subsequent lifelike reperfusion from artery to vein. European Surgical Research (In revision)

W.Willaert, K.Van Der Speeten, G.Liberale, C.Remue, A.Kartheuser, W.Ceelen. BEV-IP: Perioperative chemotherapy with bevacizumab in patients undergoing cytoreduction and intraperitoneal chemoperfusion for colorectal carcinomatosis. BMC Cancer (In Press) (impactfactor: 3.36)

F. Tozzi, **W.Willaert**, I. Van Herzeele, K. D'Herde, P.Pattyn. Systematic review of surgical training on reperfused human cadavers. (to be submitted)

A2 Peer-reviewed articles in international journals not included in the Science Citation index

Dirk Van de Putte, Yves Van Nieuwenhove, **Wouter Willaert**, Piet Pattyn, Wim Ceelen. Organ preservation in rectal cancer: current status and future perspectives. Colorectal Cancer 2015; 4(4): 185–197.

B2 Chapters in books

W.Willaert, W. Ceelen, P. Pattyn,Y.Van Nieuwenhove. Gastrointestinal Surgery Series. Colorectal surgery. Chapter 1: New insights into the surgical anatomy and embryology of the rectum: a review. Published by Jaypee Brothers Medical Publishers 2014.

W.Willaert, C.Chevallier, V.Djonov. Atlas of post-mortem Angiography. Chapter 33: The use of post-mortem angiography in clinical anatomy. Published by Springer.

C3 Conference or meeting abstracts, unpublished lectures, posters, ...

M. Bakker, K. Van Vaerenbergh, W. De Gersem, M. Coghe, T. Boterberg, C. Derie, **W. Willaert**, W. Duthoy, L. Vakaet, W. De Neve. Quality aspects and time gain of an automated procedure for generating an optimized plan in the routine treatment of breast cancer with external tangential beam irradiation. EJC Supplements Volume 1, Issue 5, September 2003. S 153-4. Poster 504. DOI:10.1016/S1359-6349(03)90536-2. (5-Year Impact Factor 5.257).

Willaert W, Ceelen W, Biglari M, de Hemptinne B. Sublay mesh repair of large midline incisional hernias with a lightweight, large pore polyglactin coated mesh. Eur Surg Res 2004; 36 (suppl1): 136. (Impact factor 1.214). Conference abstract 39th Congress of the European Society for Surgical Research, Athens, May 2004.

W.Willaert, W.Ceelen, M.Biglari, B.de Hemptinne. Sublay mesh repair of large midline incisional hernias with a light weight, large pore polyglactin coated mesh. 5th Belgian Surgical Week, Ostend, Belgium, 6th- 8th May, 2004. Conference abstract.

F.Van Praet, N.Nesher, I.Bakir, R.De Geest, I.Degrieck, F.Casselman, **W.Willaert**, H.Vanermen. Robotically enhanced minimally invasive direct coronary artery bypass surgery: an alternative surgical technique to percutaneous coronary interventions. XXIV meeting of the society of cardiac surgeons. IV Live teleconference interinstitutional . New trends in cardiac surgery, A Coruna, Spain, June 13-15th, 2005.

W.Willaert, F.Berrevoet, B.de Hemptinne, X.Rogiers, R. Troisi. A prospective randomized controlled trial shows no benefit for postoperative antibiotics after cholecystectomy for acute cholecystitis. 10th Belgian Surgical week: Ostend, Belgium, 29th April -2nd May 2009. Conference abstract.

W.Willaert. Transanal endovascular surgery. 64 ste geneeskundige dagen. Het chirurgisch geval. UZ Antwerpen, Belgium, September 18 th 2009. Conference abstract.

W.Willaert, Y.Van Nieuwenhove, T.Henckens, D.Van De Putte, K.Van Renterghem, W.Ceelen, P.Pattyn. Life-threatening side effects of malabsorptive procedures in obese patients necessitating conversion surgery: a review of 16 cases. 12th Belgian Surgical Week, Ostend, Belgium, 11th May-14th May 2011. Conference abstract.

W.Willaert, D.Van De Putte, Y. Van Nieuwenhove, W.Ceelen, P.Pattyn. *U.Z., Gent, Belgium.* Training models in laparoscopy: are they an adjunct in learning skills and surgical anatomy? 13th Belgian Surgical week, Spa, Belgium, 9-12th May 2012. Conference abstract.

W.Willaert, Y. Van Nieuwenhove, D.Van De Putte, P.Pattyn, W.Ceelen. New insights into the innervation of the levator ani muscle. 13th Belgian Surgical week, Spa, Belgium, 9th-12th May 2012. Poster.

Wouter Willaert, Tom Van Hoof, Piet Pattyn, Katharina D'Herde. Reconsidering the Thiel embalming procedure in a kidney model. 175ste vergadering van de Nederlandse Anatomen Vereniging, Lunteren, The Nederlands, 1st-2nd February 2013. Conference abstract.

Wouter Willaert, Dirk Van De Putte, Katrien Van Renterghem, Yves Van Nieuwenhove, Piet Pattyn, Wim Ceelen. Nodal counts and the extent of lymphadenectomy in colorectal cancer: a

systematic review. 14th Belgian Surgical Week, Ostend, Belgium, 1st-4th May 2013. Conference abstract.

Wouter Willaert, Dirk Van De Putte, Katrien Van Renterghem, Yves Van Nieuwenhove, Wim Ceelen, Piet Pattyn. Lifelike revascularization of embalmed kidneys: a promising new surgical training model? 14th Belgian Surgical Week, Ostend, Belgium, 1st-4th May 2013. Conference abstract.

Wouter Willaert, Dirk Van De Putte, Katrien Van Renterghem, Yves Van Nieuwenhove, Wim Ceelen, Piet Pattyn. Lifelike revascularization of embalmed kidneys: a promising new surgical training model? 48th Congress of the European Society for Surgical Research, Istanbul, Turkey, 29th May-1st June 2013. Eur Surg Res 50 (suppl 1): 62. (Impact factor 0.93). Conference abstract.

Elodie Melsens, **Wouter Willaert**, Yves Van Nieuwenhove, Stan Monstrey, Piet Pattyn. Vaginal elongation with descending colon in patients with gender identity disorder. 15th Belgian Surgical Week, Spa, Belgium, 14th-17th May 2014. Conference abstract.

Wouter Willaert, Wim Ceelen, Katharina D'Herde, Piet Pattyn. Surgical training in a revascularized soft embalmed pig model. 15th Belgian Surgical Week, Spa, Belgium, 14th-17th May 2014. Conference abstract.

Elodie Melsens, **Wouter Willaert**, Yves Van Nieuwenhove, Stan Monstrey, Piet Pattyn. Vaginal elongation with descending colon in patients with gender identity disorder. 49th Congress of the European Society for Surgical Research, Budapest, Hungary, 21th-24th May 2014. Conference abstract.

Wouter Willaert, Wim Ceelen, Katharina D'Herde, Piet Pattyn. Surgical training in a revascularized soft embalmed pig model. 49th Congress of the European Society for Surgical Research, Budapest, Hungary, 21th-24th May 2014. European Surgical Reserach. 2014, 114, (suppl 3). Conference abstract.

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