

# A cadaveric perfused model with antegrade arteriovenous pulsatile circulation: a new tool for teaching endovascular skills

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**Background:** The benefits of using cadaveric humans in surgical training are well documented, and knowledge of the latest endovascular techniques is essential in the daily practice of vascular surgeons. Our study explores the feasibility of an affordable human cadaveric model with pulsatile and heated antegrade perfusion for reliable and reproducible endovascular or surgical simulation.

**Methods:** We undertook cannulation of 7 human cadavers embalmed in a saturated salt solution to create a left-to-right central perfusion with a heated solution, from the ascending thoracic aorta to the right atrium. To that end, we used surgically created carotidjugular and femorofemoral arteriovenous fistulas. Biomedical engineers designed a prototype pump for pulsatile circulation. We monitored invasive blood pressure and temperature. We used this model for training for endovascular thoracic aortic procedures and open vascular surgeries.

**Results:** The prototype pump achieved a pulsatile flow rate of 4.7 L/min. Effective cadaveric perfusion was achieved for several hours, not only with an arterioarterial pathway but also with arteriovenous circulation. The arterial pressures and in situ temperatures accurately restored vascular functions for life-like conditions. This new model made it possible to successfully perform thoracic endovascular aortic repair, subclavian artery stenting and simulation of abdominal open vascular trauma management. The saturated salt solution method and a specifically designed pump improved cost competitiveness.

**Conclusion:** Endovascular simulation on human cadavers, optimized with the pulsatile and heated perfusion system, can be a dynamic adjunct for surgical training and familiarization with new devices. This reproducible teaching tool could be relevant in all surgery programs.

**Contexte :** Les avantages de l'utilisation de cadavres humains pour la formation en chirurgie sont bien documentés. De plus, la connaissance des dernières techniques endovasculaires est essentielle pour les chirurgiens vasculaires dans leur pratique quotidienne. Cette étude porte sur la faisabilité d'un modèle cadavérique abordable reposant sur une perfusion antérograde pulsatile et chauffée, qui permet, de manière fiable et reproductible, de simuler les conditions endovasculaires et des interventions chirurgicales.

**Méthodes :** Nous avons effectué une canulation sur 7 cadavres humains embaumés avec une solution saline saturée, le but étant de faire une perfusion centrale gauche-droite pour injecter une solution chauffée de l'aorte thoracique ascendante à l'atrium droit. À cette fin, nous avons utilisé des fistules artérioveineuses carotido-jugulaires et fémoro-fémorales créées chirurgicalement. Les ingénieurs biomédicaux ont conçu un prototype de pompe produisant une circulation pulsatile. Nous avons aussi fait un monitoring endovasculaire de la pression et de la température. Ce modèle a servi pour de la formation sur les interventions endovasculaires dans l'aorte thoracique et sur les chirurgies vasculaires effractives.

**Résultats :** La pompe assurait un débit pulsatile de 4,7 L/min. Une perfusion a été réalisée sur les cadavres pendant plusieurs heures, non seulement par voie artério-artérielle, mais aussi avec circulation artérioveineuse. Les pressions artérielles et températures in situ obtenues ont permis de recréer avec exactitude les fonctions vasculaires d'une personne vivante. Ce nouveau modèle a permis de réaliser avec succès une réparation endovasculaire de l'aorte thoracique, l'implantation d'une endoprothèse dans l'artère subclavière et la simulation d'une prise en charge d'un trauma vasculaire effractif dans l'abdomen. L'emploi d'une solution saline saturée et d'une pompe conçue sur mesure a permis de mener l'exercice à coût avantageux.

**Conclusion :** La simulation endovasculaire sur des cadavres humains, optimisée grâce à un système de perfusion pulsatile et chauffée, peut être un procédé complémentaire dynamique pour la formation chirurgicale et la familiarisation avec de nouveaux appareils. Cet outil d'enseignement reproductible pourrait être utile dans tous les programmes de chirurgie.

**T**raining and education in surgical specialties require a complete, structured program with adequate exposure to various procedures for the trainees. However, evolving techniques and work-hour restrictions for residents limit the development of surgical skills. In addition, the preeminent place now occupied by endovascular surgery reduces the exposure of young surgeons to open vascular surgery.<sup>1</sup> Therefore, the need for a model to train future surgeons, especially for the acquisition of more experience and expertise without extension of the residency training period, has become more relevant than ever.<sup>1</sup>

Hands-on programs, such as laboratory testing, are being developed<sup>2</sup> and many simulation programs are currently available, including bench models, live animals and cadavers. New techniques are also accessible, such as virtual reality surgical simulators.<sup>2</sup> Nonetheless, cadaver simulation remains the best option to reproduce true-to-life vascular conditions with a high degree of fidelity. However, its cost and availability are major limiting factors for their use.<sup>3</sup>

Anatomic dissection is part of many medical training programs worldwide and is an essential tool for trainees to grasp new techniques and improve skills.<sup>4</sup> In addition, medical academies increasingly favour the use of simulation for health safety and to optimize ongoing medical and surgical training.<sup>5</sup>

Vascular surgery training comes with specific requirements because the subject matter is a dynamic organic system with specific physical constraints, in both open and endovascular surgery.<sup>1</sup> Many simulation tools have been developed and are currently used (e.g., computer simulators, test benches, mannequins, animal cadavers, live animals, standard human cadavers), but these are limited when it comes to realistic vascular pulsatility, tissue integrity, tactile sensations, cost and accessibility.<sup>6</sup> Moreover, virtual simulators do not confer the same tactile feedback as cadaveric simulations.<sup>7</sup>

Access to perfused cadaveric models is very uncommon in the medical world. Although virtual and cadaveric simulation models are currently on the market, their accessibility is limited by several factors, especially their cost. For example, the model developed by Delpuch and colleagues<sup>8</sup> costs about €80 000 for a single training unit. Of the few cadaveric models currently described, one that offers pulsatility, a realistic blood pressure range and arteriovenous flow has yet to be presented. In view of this, our main objective was to develop a pulsed and heated cadaveric model with antegrade arteriovenous perfusion that is affordable, reliable and capable of reproducing life-like surgical conditions for training and educational purposes.

## METHODS

### *Preservation and preparation of salt-embalmed cadavers*

We prepared the cadavers according to laboratory protocol. All the hair was shaved and the bodies were washed with water and an antiseptic liquid (DETTOL, Reckitt Benckiser). Subsequently, we flushed the vascular system with the infusion of a chemical compound (Proflow, Dodge Chemicals). Once the catheters were positioned, we flushed the right common carotid and superficial arteries by cannulation with a pump.

We chose to use cadaveric bodies embalmed and stored in an adapted saturated salt solution (SSS) for this study. Typically, in the laboratory at the Université du Québec à Trois-Rivières (UQTR), corpses with a body mass index of 23–25 are embalmed with SSS to help preserve the firmness of the vessels. The time between embalming and availability for use is a minimum of 6 weeks. For each cadaver, a total of 25 L of solution was prepared, containing 20 kg of sodium chloride, 1 L of formaldehyde solution (20%), 200 mL of phenol, 500 mL of glycerol, 4 L of isopropyl alcohol and 19.3 L of water.<sup>9</sup>

### *Cadaver selection*

We selected cadavers according to the following criteria: no known vascular history, no major or minor vascular surgery and no history of intraperitoneal cancer or known tumour that could affect vascular condition or access.

The open surgical and aortic endovascular procedures took place between March 2019 and January 2020. To preserve donor anonymity, the identity and medical history of the subjects were not disclosed.

### *Surgical and infusion preparation*

To achieve arteriovenous circulation, peripheral carotidojugular and femorofemoral arteriovenous fistulas (Figure 1), as well as central arterial and venous cannulation, were performed as described below. We sealed the anastomoses with a cyanoacrylate-based glue (Dermabond, Ethicon) when needed.

### *Femoral approach*

We exposed the common femoral arteries and femoral veins bilaterally through a longitudinal inguinal incision. These access points were the same as those used during cadaver preparation.

We created the arteriovenous fistulas by an end-to-side anastomosis in 1 of 2 ways: either between the distal

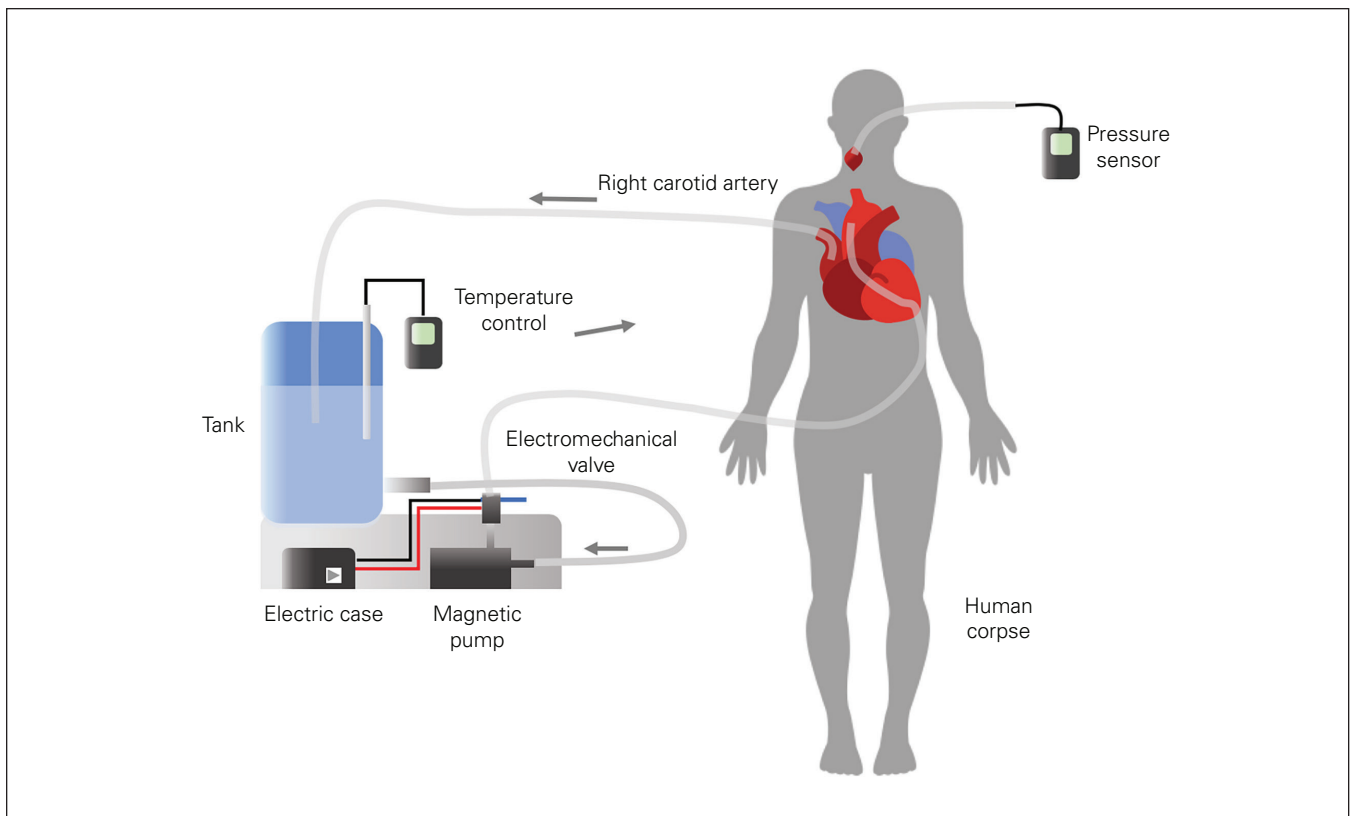


Fig. 1. Schematic drawing of the cadaveric circulatory system with the prototype pump.

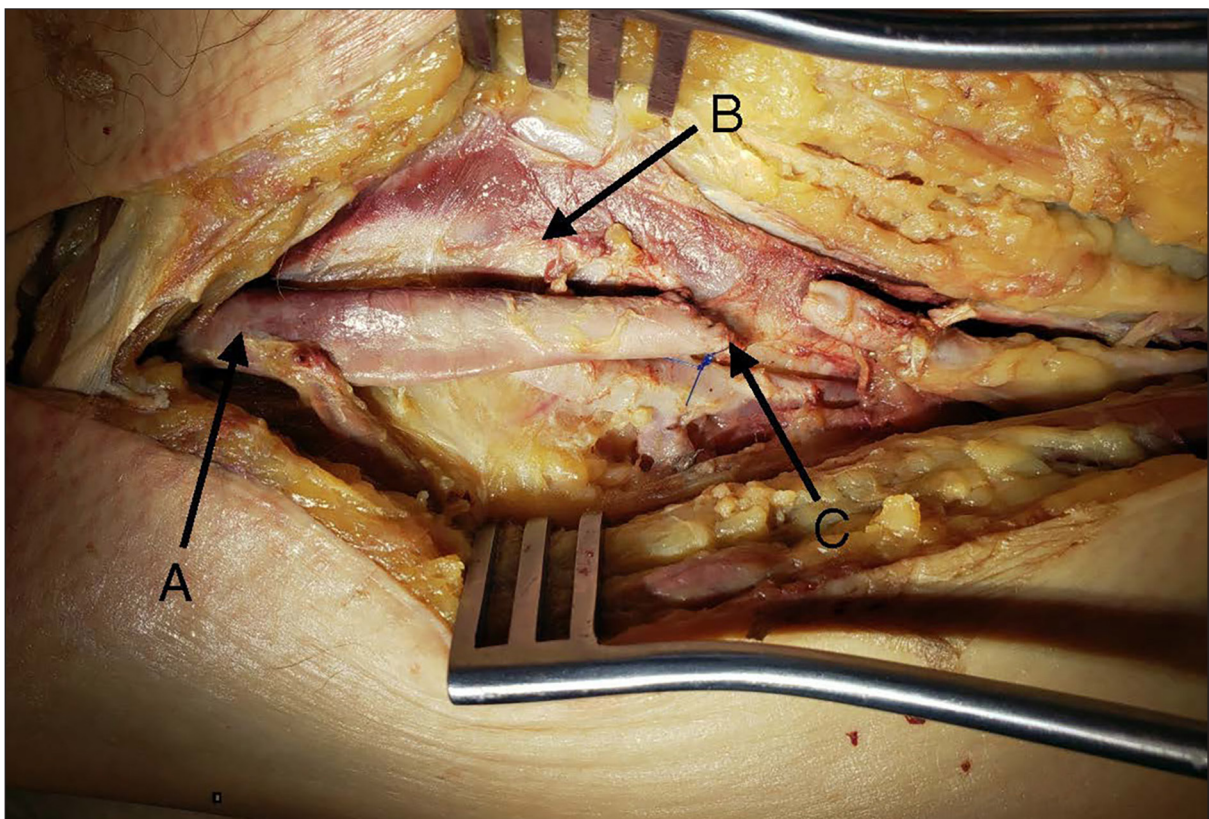
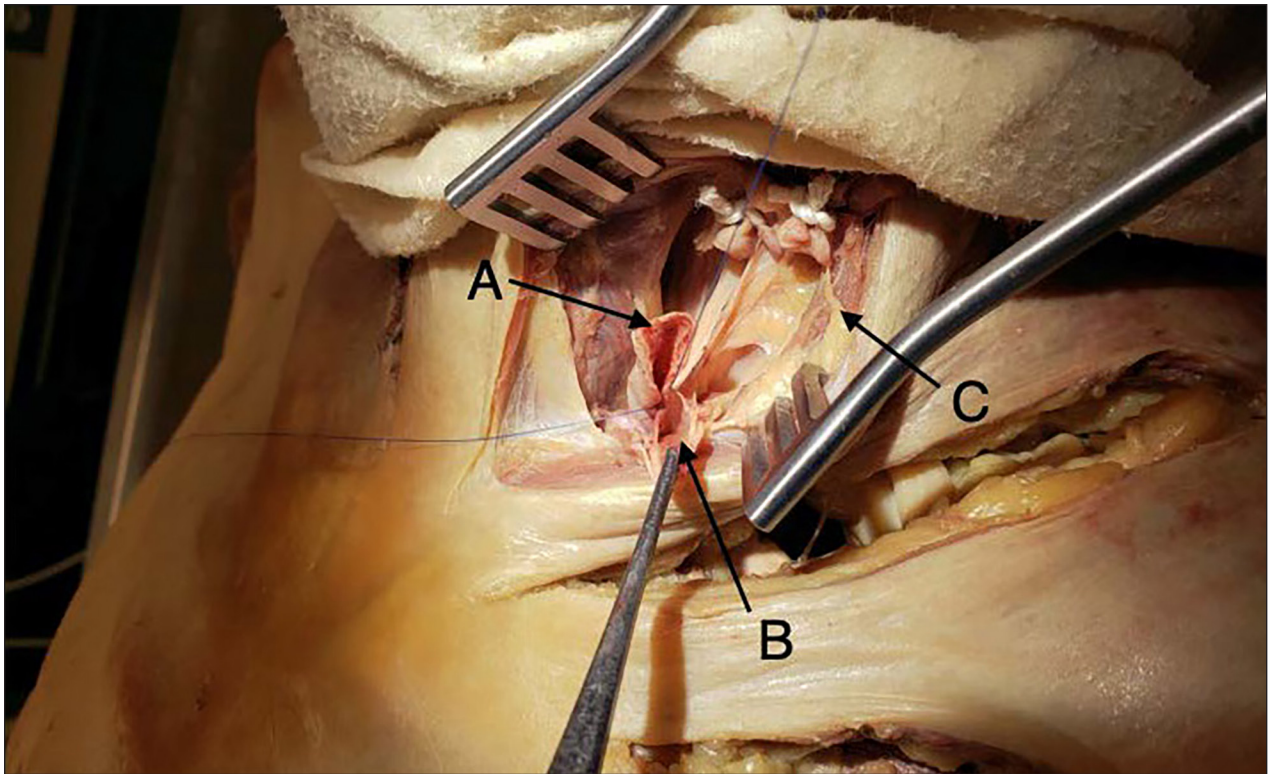


Fig. 2. The femorofemoral arteriovenous fistula. Note: A = common femoral artery, B = common femoral vein, C = arteriovenous anastomosis. Photograph by Guillaume Febrer.



**Fig. 3.** The carotidojugular arteriovenous fistula. Note: A = common carotid artery, B = internal jugular vein, C = sternocleidomastoid muscle. Photograph by Guillaume Febrer.

common femoral artery and the common femoral vein, or between the superficial femoral artery and the deep femoral vein, to free the common femoral artery. We used 5–0 prolene suture, followed by downstream arterial ligation and upstream venous ligation (Figure 2).

#### *Carotid approach*

We exposed the common carotid arteries and internal jugular veins bilaterally through a longitudinal cervical incision. We created the arteriovenous fistulas by an end-to-end anastomosis between the distal common carotid artery and the internal jugular vein with a 5–0 prolene suture, followed by downstream arterial ligation and upstream venous ligation (Figure 3).

#### *Preparation of central left-to-right infusion*

A full sternotomy provided access to the cardi thoracic structures. We exposed the right atrium and the ascending thoracic intra- and extrapericardial aorta. Venous cannulation was done in the right atrium via the right auricle. We secured the cannula with a 0-gauge silk purse-string suture.

We undertook arterial cannulation in 3 ways: transapical cannulation (2 cadavers) with a 7 mm (Edwards EZ-GLIDE EZS21A 21F) intubation probe, direct cannulation of the ascending aorta (3 cadavers) and indirect cannulation

(2 cadavers) using a Dacron knitted double velour microvel vascular graft (Hemashield Gold, Maquet). These different approaches allowed us to test the techniques reported in the literature and help assess their applicability in our model.<sup>10</sup>

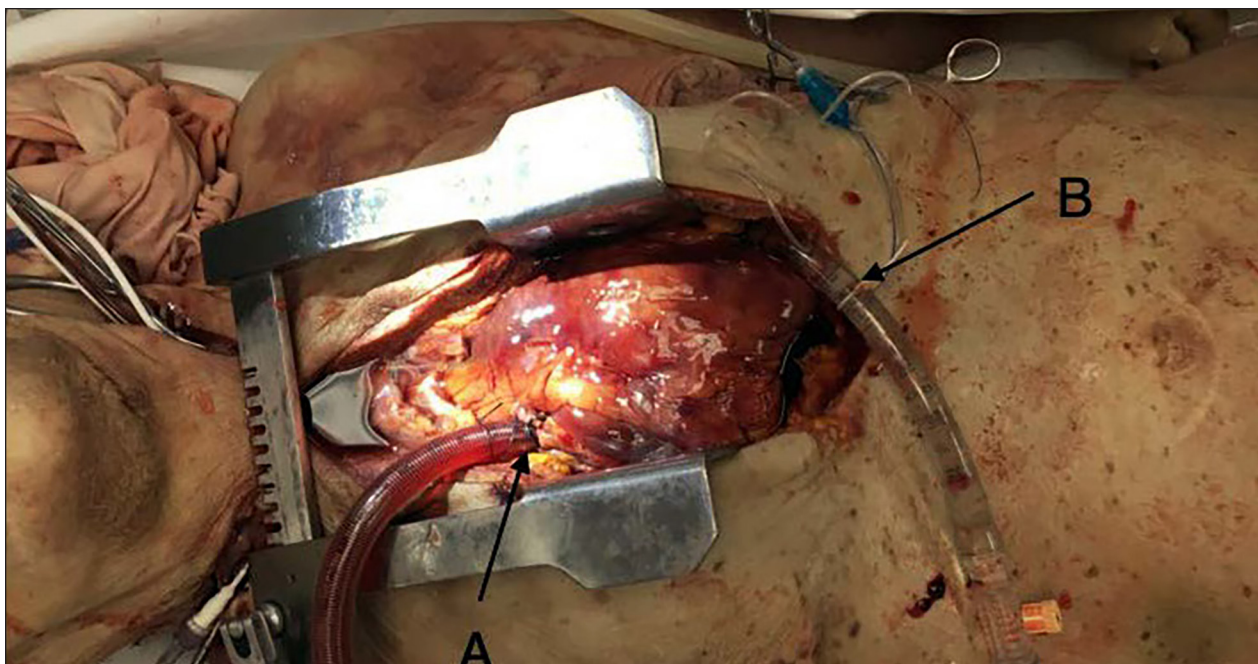
We positioned the purse-string suture threads at the level of the right auricle and the apex of the left ventricle (Figure 4). We inserted central perfusion cannulas through these 2 cavities.

For the first approach, the endotracheal tube, positioned at the root of the ascending thoracic aorta via the apex of the left ventricle, supplied the arterial flow. The balloon was inflated to seal the system in the ascending aorta. The second approach was performed using direct cannulation through an arterial cannula with a purse-string suture on the ascending aorta. The third approach used prosthetic graft cannulation with a lateral anastomosis on the ascending aorta.

The cannula, positioned in the right atrium (CardioMed CM-6736V [36 French]), made it possible to collect the flow from the venous return. The cannulas were connected to the perfusion system and then purged to evacuate any clot present in the vessels before connecting and closing the perfusion circuit.

#### *Perfusion system*

Our engineering team designed the perfusion system (LifeEngine) to generate a pulsatile physiologic blood



**Fig. 4.** Central left-to-right infusion via (A) the left ventricle and (B) the right auricle. Photograph by Guillaume Febrer.

pressure by perfusing the corpse with a heated solution (Figure 1). The infusion solution was made of 1 kg of gelatin (Sigma–Aldrich), dissolved in 20 L of water and 1 kg of salt. To regulate the initial flow rate and increase it progressively, a manual valve was positioned at the outlet of the container, upstream of an electromagnetic pump (MP-15MR-110, Sisan Pump). The pump was then connected to a solenoid valve (2W-160-15, Adafruit) and monitored by a microcontroller (Photon, Particle) to induce a pulsatile flow. We perfused the cadaver with the pulsed fluid through an inlet cannula, as described in the previous section. The outflow was retrieved, filtered and returned into the reservoir. A thermal generator was immersed in the perfusion solution to generate a temperature of 38°C, and to then maintain a temperature between 36°C and 37°C for the perfused liquid. Thirty minutes are needed for the perfusion liquid to reach physiologic temperature. The system is reusable, with only a thorough rinsing needed after each procedure (pump, tubing, cannulas). The endovascular material (guide, balloon) will eventually need to be changed once it becomes defective.

#### *Pressure, pulsatility and temperature monitoring*

We monitored continuous blood pressure with arterial catheterization of the carotid, subclavian and femoral arteries (Arrow Seldinger Arterial Catheter, USA Teleflex), connected to the pressure sensor of a transport monitoring system. The video recording obtained was copied through an external DVI2USB capture device (Epiphan Systems). The pump controls pulsatility through a number of different factors, including flow rate of liquid, pulsation rate (number

of pulses per minute) and the ratio of systolic (high pressure) to diastolic (low pressure) duration. The site where the fistula is created helps improve angiographic views of a given vascular area. Partial or full clamping of 1 of the fistulas will increase peripheral vascular resistance and improve perfusion pressure. Self-expanding stents, balloon-mounted stents and thoracic aortic stent grafts (Captivia, Medtronic, Zenith and Cook) were deployed.

#### *Imaging*

We positioned the studied subjects on a radiolucent table. We performed angiograms using a mobile C-arm (Veradius Unity, Philips) with iodinated contrast (Visipaque 270 mg/mL), injected through a pigtail catheter. Visualization of the arterial vascular tree was recorded by digital subtraction angiography during the procedures.

#### *Ethics approval*

We developed this model with the agreement of the Université du Québec at Trois-Rivières (UQTR) ethics committee (SCELERA-19-03), as well as the Hôpital du Sacré-Coeur de Montréal ethics committee (SCELERA 2020-2079). Permission to take photos and videos was obtained from the UQTR anatomy laboratory.

#### **RESULTS**

We included 7 cadavers from the anatomy laboratory of the UQTR. The procedures and arterial cannulation techniques were successfully completed.

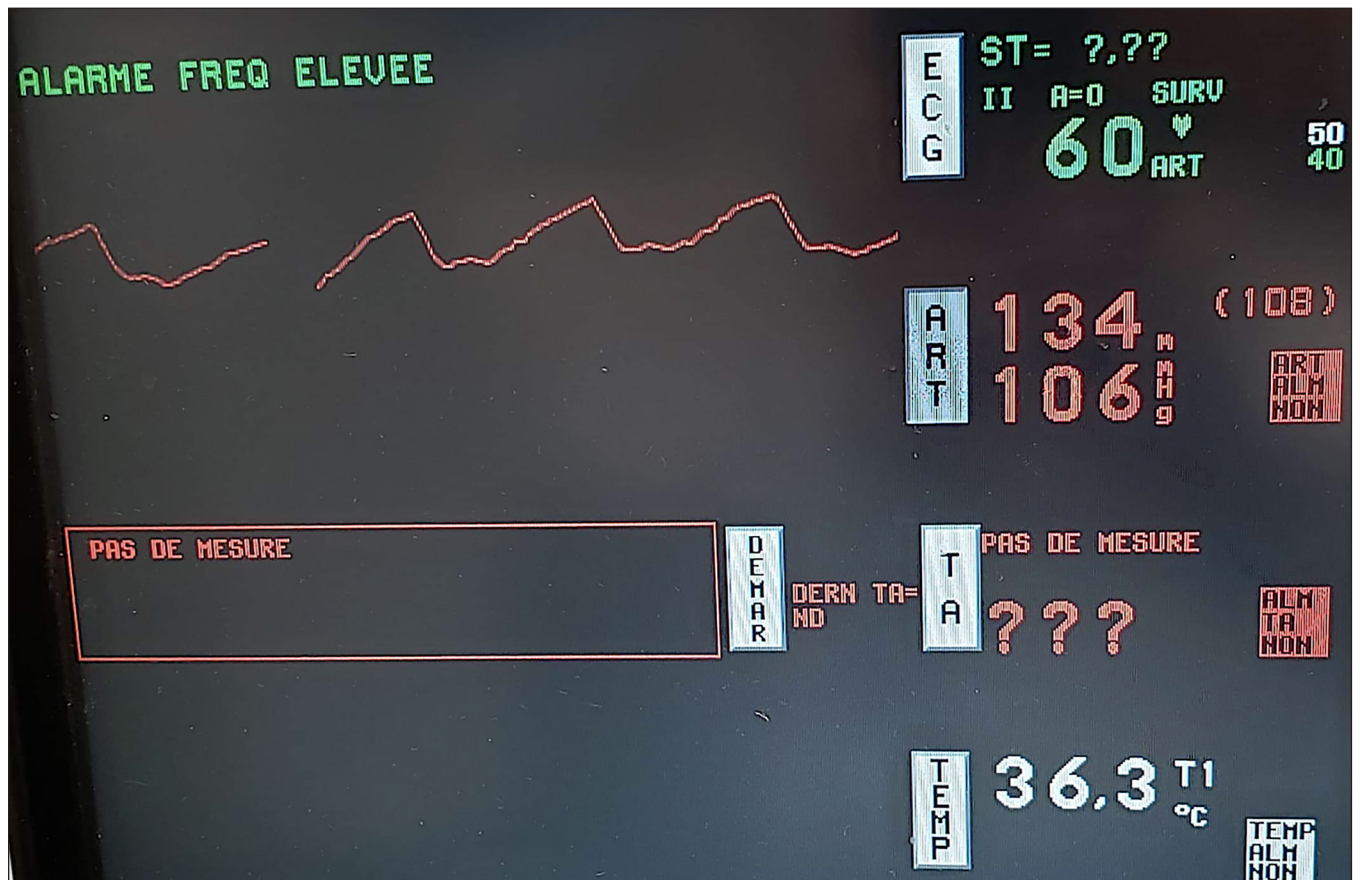


Fig. 5. Monitoring of carotid arterial pressures and of the temperature at the esophagus. Photograph by Guillaume Febrer.

### Technical achievement

Variations in arterial pressure were made possible by modulating fistula size, with a smaller size yielding a higher perfusion pressure. We obtained an average systolic pressure of 120 (80–170) mmHg and a diastolic pressure of 80 (40–110) mmHg. A constant flow rate of 4.7 L/min was maintained, to reproduce a realistic pulse pressure (Figure 5).

Depending on the cadaver, an esophageal temperature of 35°C–38°C (mean 36.8°C) was achieved.

### Multi-organic permeability and pulsatility

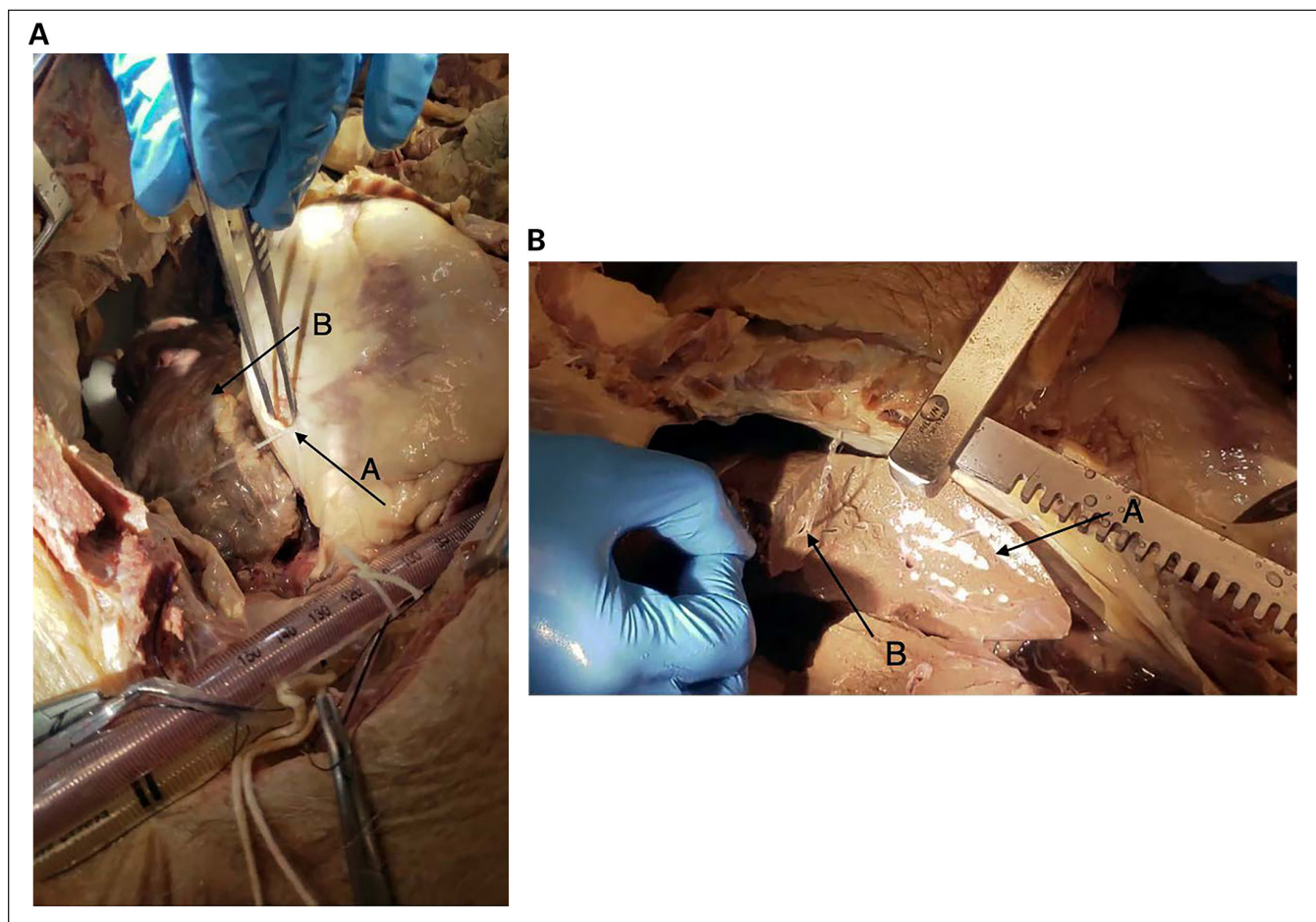
We observed arterial patency throughout the arterial vascular tree, including small arteries. The pulse was palpable on the aorta and the main arterial axis. Sections of the hepatic parenchyma, via the transperitoneal abdominal route, showed the transmission of pulsatility to the arteries of the abdominal wall and to the intrahepatic arteries. This was also seen in the splenic parenchyma, the mesentery, the renal parenchyma and the anterior interventricular artery (Figure 6). This model could be used for surgeries considered bloody, such as liver resection or splenic repair, as we saw the vascular permeability of the hepatic, renal and cardiovascular systems

(Figure 6A). The body preservation method needs to be considered to achieve the highest degree of realism. We did not attempt the creation of more distal fistulas. However, isolated perfusion of a lower limb showed the lack of transcapillary exchange without venous return.

### Stent deployment simulation

In the first scenario, we aimed to simulate the endovascular exclusion of an aortic lesion, such as a ruptured sacular aneurysm, by placing a thoracic aortic stent (Zenith Alpha, Cook) in the descending thoracic aorta. This procedure was performed via the right femoral approach using a Lunderquist guide, according to standard clinical practice, and completed by inflation of a Coda balloon (Cook) via an introducer (DrySeal sheath). Angiographic control, performed using a pigtail probe, was satisfactory, with proper positioning and deployment of the stent (Appendix 1, available at [www.canjsurg.ca/lookup/doi/10.1503/cjs.023020/tab-related-content](http://www.canjsurg.ca/lookup/doi/10.1503/cjs.023020/tab-related-content)).

A second scenario aimed to simulate endovascular exclusion of the aortic arch and of a descending thoracic aortic aneurysm. These pathologies were addressed by placing a surgeon-modified fenestrated stent (Valiant, Medtronic) with a window facing the left subclavian



**Fig. 6.** Arterial pulse for the (A) anterior descending artery ([A] arrow shows descending artery; [B] arrow shows the right atrium) and the (B) left hepatic lobe ([A] arrow shows liver pachydermia and [B] arrow shows the hepatic artery branch). Photographs by Guillaume Febrer.

artery ostium and another stent (Atrium) via the left humeral approach (Appendix 2, available at [www.canjsurg.ca/lookup/doi/10.1503/cjs.023020/tab-related-content](http://www.canjsurg.ca/lookup/doi/10.1503/cjs.023020/tab-related-content)). The modified fenestrated stent preparation was carried out according to the technique described by Canaud and colleagues.<sup>11</sup>

We successfully deployed a self-expanding stent and a balloon-mounted stent in 2 of the 7 cadavers.

### Cost analysis

The first prototype, created with material that was available to anyone and can be found everywhere, cost about US\$392 (Table 1). We estimate that the final cost of the model, of industrial design and including specialized components designed for adequate performance, would cost less than Can\$15 000.

### DISCUSSION

This perfused and heated cadaver model with antegrade pulsatile perfusion would allow training workshops with

**Table 1. Cost analysis of the perfusion prototype**

Item	Cost, US\$*
Pump	70.83
Solenoid valve	34.68
Flowrate sensor	23.78
Temperature controller	43.67
Electric components, power supply and electric protection supply	82.73
Fluidic system parts, tubing and tank	136.23
Total	391.92

\*Cost for a cadaver embalmed by saturated salt solution is US\$700.

a high degree of realism. Various attempts to develop cadaver models have been described. These were reviewed by Bellier and colleagues,<sup>12</sup> who defined 6 performance criteria for cadaver simulation. Our model with salt bodies achieved 5 of the 6 criteria (pulsatility, coloured perfusion liquid, perfusion of the aortic arch, room temperature, satisfactory arterial pressure), equal to the SimLife models<sup>8</sup> and the model presented by Aboud and colleagues.<sup>13</sup> The only criterion not completely

achieved with the SSS method was ventilation, which is possible but limited, given low compliance of the respiratory system. Ventilation, however, is compatible with the Thiel model.<sup>14,15</sup>

All of the models described by Bellier and colleagues<sup>12</sup> can be used for endovascular simulation. However, only a pressurized model like ours can also be used to simulate surgical reconstructions in open vascular reconstruction or in traumatology. Indeed, the Delpech<sup>8</sup> and Aboud<sup>13</sup> models do not provide continuous pulsatile arteriovenous circulation. Our model was designed to be versatile, but in terms of simulation, a purely cervical cannulation will be closest to real-life conditions.

We chose to have a left-to-right surgical cannulation to simulate the cardiopulmonary bypass circuit used in cardiovascular surgery, aiming to reproduce real-life conditions as closely as possible. The anastomoses could be carried out in less than an hour by 2 trained vascular surgeons, and this process could be a relevant educational tool. Two other types of infusions are reported in the literature, but these were not carried out in our model. The left-to-left perfusion, as illustrated by the model developed by Garrett,<sup>16</sup> transfers liquid from 1 main arterial vascular axis to another and collects it in dedicated containers. It is faster to implement, but the drawback is the absence of venous pressure. Alternatively, independent systemic venous and arterial perfusion can be implemented, allowing simultaneous antegrade arterial flow and retrograde venous flow via independent cannulas, as seen in the models developed by Delpech and colleagues,<sup>8</sup> and Aboud and colleagues.<sup>13</sup> These have the advantage of pressurizing the arterial and venous systems quickly and providing adequate simulation; however, cannulation techniques, as performed in everyday cardiovascular surgery, are not possible in these models.

Our choice of SSS-embalmed cadavers was motivated by their affordability and ease of use, compared with fresh frozen bodies. Indeed, fresh frozen bodies need to thaw for 36–72 hours before each use. In addition, even if several serological tests are performed before the cadaver is frozen, there is always a risk of contamination and infection that the salt-embalmed cadaveric model does not present.<sup>17</sup> Therefore, preparation time is shorter and safer. Also, the SSS method does not require specific structures or very sophisticated installations. Cadavers can be stored at room temperature without any risk of putrefaction or loss of viability.

Finally, SSS-embalmed cadavers are ideal for vascular and endovascular simulations since tissue integrity, including the appearance and texture of the vessels, is preserved. The life-like appearance of the tissues provides a very realistic experience for new surgeons.<sup>18</sup> Moreover, the loss or leakage of fluid into the organs is minimal, helping to prevent tissue distraction caused by the extravasation of fluids and the resulting modifications to the anatomic structures.

Our model, like that of Arbatli and colleagues,<sup>10</sup> provides an ideal temperature for endovascular manipulations. Indeed, Robich and colleagues<sup>19</sup> have shown that stents, especially those containing nitinol, were deployed and handled more easily in an environment with a temperature above 30°C.

Overall, our model is optimal for educational purposes, as well as for residents training in surgery programs. Specifically, it features the vascular anatomic variations, as well as the realistic visual and tactile sensory perceptions, found in real-life surgical conditions. This is of paramount importance for the trainee. Indeed, according to Adams' principle of the psychomotor feedback loop, having a good sensory stimulus improves interpretation and recognition for the learner.<sup>20</sup> The feedback loop has the advantage of being dynamic, allowing the student to adapt in a matter of seconds, letting him perfect his knowledge, both technical and theoretical.

Furthermore, new and complex endovascular techniques are constantly emerging and require a learning curve before they can be mastered. Our model, like those of Aboud and colleagues,<sup>13</sup> and Delpech and colleagues,<sup>8</sup> allows vascular surgeons and interventional radiologists, as well as residents in training, to practise these new techniques. Our model achieved continuous arteriovenous circulation with pulsatility and in situ realistic physiologic arterial pressures and temperature. It also allowed us to successfully perform complex endovascular procedures, such as the placement of a thoracic aortic endograft and iliac artery stent deployment, accurately and in realistic conditions. This model represents a reliable simulation tool for emerging or established endovascular techniques, as well as general or vascular open surgery. In addition, the constant antegrade arteriovenous flow and pulsatility offer a natural contrast flow during endovascular interventions, adding technical realism to our model.

Aboud and colleagues<sup>13</sup> successfully implemented surgical workshops for residents. The cadavers were prepared to simulate different surgical emergencies, such as open limb or even skull fractures. This allowed trauma surgeons to experience emergency situations while working in an environment that was less stressful and with less imminent risk, giving them the opportunity to perform the necessary surgical procedures properly. Similarly, our model could be used in workshops for vascular emergencies such as ruptured abdominal aortas.

The main advantage of our model over the SimLife model is its accessibility because of its substantially lower cost. The SimLife model equipment is about €80 000 for an average of 100 uses, or €800 per use.<sup>8,21</sup> We estimated that our model would cost less than Can\$15 000, which improves cost effectiveness and competitiveness.



Some institutions prefer using fresh cadaveric bodies, for a slight reduction in costs when it comes to preparation. Nonetheless, SSS remains the simplest, cheapest and most easily accessible among the different cadaver preservation methods.<sup>18,22</sup> As opposed to fresh cadavers, SSS embalmed corpses can be used and reused over a long period of time.

### Limitations

After flushing the vessels with the perfusion solution, residual intra-arterial thrombi that could induce artifacts can be present; a solution could be to add a specific lavage preparation step to dissolve the thrombi further. Some groups, like Delpéch and colleagues,<sup>8</sup> eliminated the remaining thrombi by using heparin in addition to a water-based washing liquid.

The set-up for each circulatory model requires a substantial time investment (90 min) or the collaboration of several teams. Indeed, collaboration between physicians and engineers is essential to ensure that such a model works properly.

The arteriovenous fistulas cause a deformation at the level of the vascular access, condemning these vessels and limiting future manipulations. Moreover, only 1 stent can be deployed in the same anatomic location since it will be very difficult to remove once deployed, with a high risk to tissue integrity.

Rigidity is another consequence of preserving bodies in SSS. This can prevent some procedures, such as airway intubation or easy mobilization of the neck or certain important joints (e.g., the knee). Thiel's embalming technique is a very good alternative when these kinds of procedures are desired.<sup>14</sup> However, Thiel's method leads to substantial tissue extravasation during perfusion, which can complicate handling.<sup>23,24</sup>

### CONCLUSION

Endovascular simulation on human cadavers, optimized with this system, can play an important role in the modern dynamics of surgical training. This pulsatile anterograde model with continuous and heated arteriovenous flow is an important contribution to surgical training in Canada. This inexpensive, reproducible educational tool could be relevant for surgical simulation, especially for vascular surgery, endovascular interventions and visceral surgery.

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**Competing interests:** Ahmad Rifahi and Elena Refet-Mollof report patents that are broadly relevant to this study. Catherine Forest-Nault and Aymeric Guy are cofounders and co-owners of LifeEngine Technologies, the company that designed this system, and hold the patent for the system. No other competing interests were declared.

**Contributors:** All authors contributed to the conception and design of the work. Ahmad Rifahi, Robin Chastant, Catherine Forest-Nault, Elena Refet-Mollof, Aymeric Guy, Emmanuel Charbonney and Guillaume Febrer contributed to the acquisition, analysis, and interpretation of data. Ahmad Rifahi and Elena Refet-Mollof drafted the manuscript. All authors revised it critically for important intellectual content, gave final approval of the version to be published and agreed to be accountable for all aspects of the work.

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