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ORIGINAL ARTICLE

Transforming orthotopic liver transplantation: Innovative dry-lab simulation model in mice ☆

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Introduction

The use of animal models is crucial for the advancement of medical research, especially for complex procedures such as liver transplantation, which are essential for understanding pathophysiological processes and underlying mechanisms in mice and provide a foundation for translating knowledge into clinical practice. Despite innovations such as *in vitro* organoid systems, organ-on-a-chip models, advanced spatial sequencing tools, and artificial intelligence (AI)-driven simulations - which have made certain animal experiments redundant - critical insights into multiorgan interactions and complex immunological mechanisms underlying liver transplant tolerance and rejection necessitate continuous, use of animal models.[1-3] Liver transplantation remains the definitive treatment for end-stage liver disease, but the nuances of early graft rejection and the physiological basis of post-transplant organ dysfunction remain incompletely understood.[4,5] However, ethical implications and the considerable number of animals required during the learning curve of the procedure raise significant concerns.[6]

To reduce the use of laboratory animals, we propose refinement of the traditional training paradigm by developing simulation models for dry-lab use. The mouse liver transplant model we have designed simulates the live animal surgical procedure and aims to significantly reduce the number of animals used during preliminary training.[7] The overall aim of this simulation process is to allow trainees to acquire the necessary skills by providing step-wise milestone-driven training, with the ultimate goal of achieving high surgical success rates in live animal testing.[8] Microsurgical training courses for complex and delicate anastomoses also utilize ro-

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dents for training purposes.[9] This model can similarly be used to train for small-diameter vessel anastomoses in confined spaces, as needed in rodent transplantation or pediatric liver transplantation, without being reliant on live animals with their ethical, organizational, and financial considerations.[10]

The described simulation model includes a detailed 3D liver and hilar structures (made of silicone foam and latex) that are inserted into a mouse skeleton generated from a CT scan and is 3D printed (polylactic acid). This allows students, residents, microsurgeons, pediatric transplant surgeons, and veterinarians to practice complex surgical techniques, such as vascular anastomoses, which are crucial for liver transplantation, in a controlled environment that simulates the operating scenario.

Incorporation of this simulation model into a research training program could potentially reduce the numbers of animals used for experimental approaches regarding murine liver transplantation and microsurgical training for pediatric liver transplantation. Furthermore, this approach reduces ethical concerns associated with the use of animals for microsurgical training purposes, as trainees are less inexperienced after practicing on a realistic model and more familiar with the procedure before first performing the technique on live animals. Ultimately, this can improve the quality of the scientific data obtained from the required animal testing and optimize animal experimental approaches in regards of refinement, reduction, and replacement (3R).

Here, we present our simulation model for orthotopic liver transplantation in mice using cuff and stent anastomoses, as well as continuous suture anastomoses. The *in vivo* procedure has been described previously by different groups.[11-14] This novel dry-lab mouse model has the potential to both facilitate training and reduce animal use.

Materials and methods

Surgical equipment

A Leica M620 TTS tabletop surgical microscope (Leica Microsystems, Wetzlar, Germany), B.Braun, FST, Mitaka and Roboz microsurgical tools (scissors, forceps, and needle holder), and suture material (silk 7-0 and nylon 10-0) were used as the basic microsurgical equipment (Fig. 1A-C).

Creation of 3D-models of the mouse, liver, vessels, and bile duct

We used Perkin-Elmer FX micro-CT (computed tomography) to create whole-body scans of a standard mouse (Fig. 2A-B), which is the liver schematic. We used the Medical Imaging and Interaction Toolkit (MITK, German Cancer Research Center, Heidelberg, Germany, www.mitk.org) to segment the liver and the bony structures of the mouse to create 3D-models of them (Fig. 2B-C) and to measure the diameters of the supra- and infra-hepatic inferior vena cava (Fig. 2D-E), portal vein (Fig. 2F), and hepatic artery. The liver and skeleton models were post-processed using MeshMixer (Autodesk, Inc., CA, USA) to correct for mesh errors and to make the model easily printable.

Artificial vessel production

Artificial vessels with the respective diameters (Fig. 3A) were created by using a natural latex mixture (Liquid Latex, Mehron, Inc. NY, USA; Latexmilch, Natursache, Germany). The latex mixture was colored using a food coloring agent, and intravenous line catheters with corresponding diameters were dipped in the mixture and left to dry. After that, the catheters were peeled off

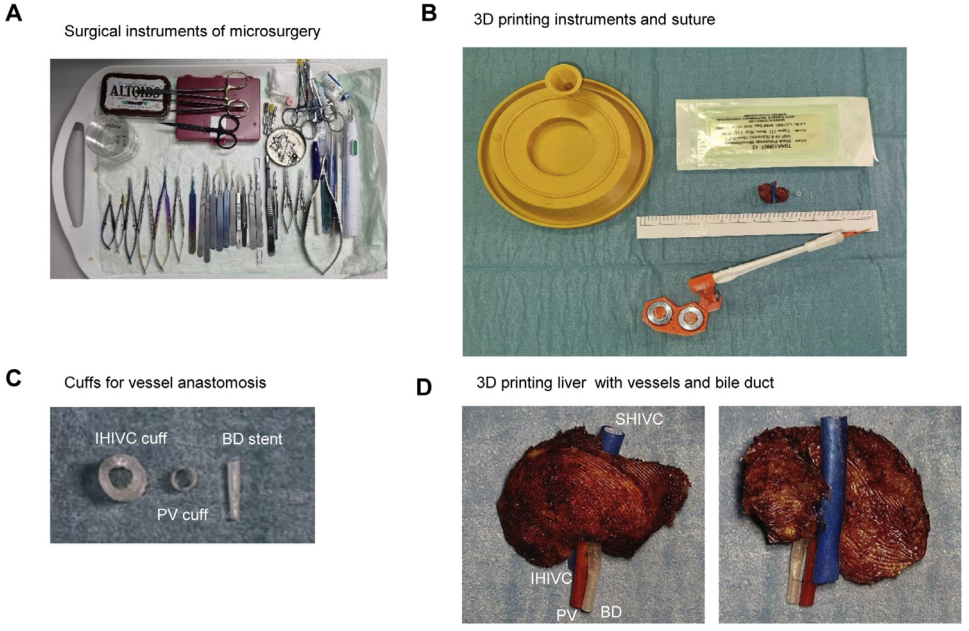


Fig. 1. Simulated liver with vessels and bile duct for training liver transplantation in mice. (A) Surgical instruments for microsurgery; (B) 3D-printed retraction devices for mouse liver transplantation; (C) Vessel cuffs for the different vessels and bile duct. (D) Simulation of mouse liver with suprahepatic inferior vena cava (SHIVC), infrahepatic inferior vena cava (IHIVC), portal vein (PV), and the bile duct (BD).

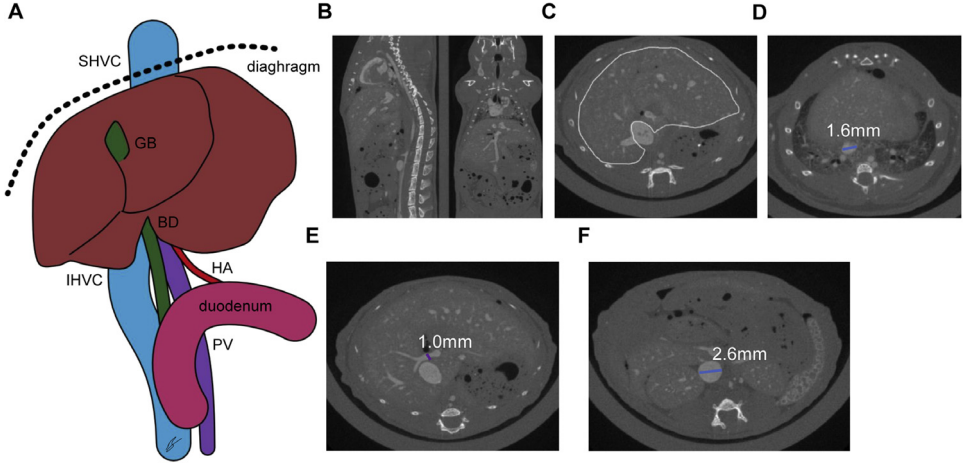


Fig. 2. Vessel models derived from murine CT scans. (A) Diagram of the mouse liver showing the suprahepatic inferior vena cava (SHIVC), the infrahepatic inferior vena cava (IHIVC), the portal vein (PV), and the hepatic artery (HA), as well as the bile duct (BD) and gallbladder (GB). (B) CT of a mouse depicting thoracic and abdominal organs. (C) Segmentation of the murine liver. (D) Measurement of the SHIVC. (E) Measurement of the PV. (F) Measurement of the IHIVC.

A Vessel diameters in the mouse and their catheter equivalent for production.

Vessel	mm	French	Gauge
Suprahepatic V. cava	1.60	5	16 1.652 mm outer diameter
Infrahepatic V. cava	2.6	8 2.66mm outer diameter	12.3
Portal vein	1.00	2.7	20 1.1mm outer diameter
Bile duct	n/a	2.1	22G 0.718 outer diameter

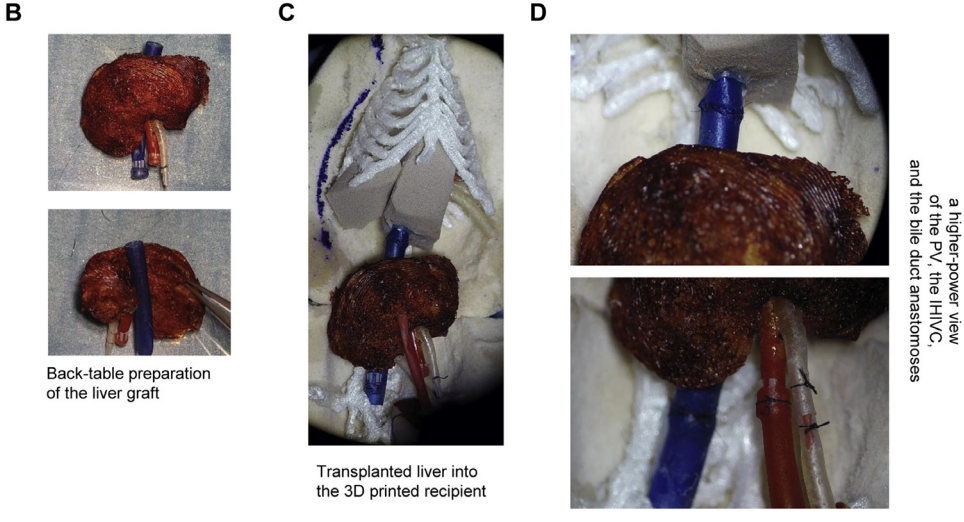


Fig. 3. Simulated mouse for training orthotopic liver transplantation. (A) Vessel diameters in the mouse and their catheter equivalent for production. (B) Back-table preparation of the simulation liver with cuffing and ligation of the infrahepatic inferior vena cava (IHIVC), the portal vein (PV), the hepatic artery (HA), and stent insertion and ligation of the bile duct (BD). (C) Transplanted murine simulation liver in the 3D-printed recipient mouse with a suture anastomosis of the suprahepatic inferior vena cava (SHIVC) and cuff-anastomosis of IHIVC, (PV), and HA, and stent anastomosis BD. (D) A higher-power view of the SHIVC, PV, IHIVC, and BD anastomoses.

the mold and trimmed. The quality of the vessels was checked under the microscope. Vessels free of perforations, with a consistent wall thickness and good flexibility, were selected.

Artificial liver production

The segmented liver model was used to create liver molds for the silicone pouring process (MeshMixer Autodesk, Inc., CA, USA). The models were 3D-printed using polyactid acid and polyvinyl ether on a 3D-printer via double-extruding for each (UltiMaker, S5, Utrecht, Netherlands) with the help of the University Center for 3D printing at the University Medical Center Hamburg-Eppendorf. To create the liver models, we used the polyurethane foam FlexFoam-iT III in the molds (Smooth-On, Inc., PA, USA). After curing, the 3D-models were extracted and painted brown using a permanent marker. We glued the portal vein, retrohepatic vena cava, and bile duct to the artificial liver under the microscope using a cyanoacrylate glue (Fig. 1D).

Artificial mouse body production

We used the Medical Imaging and Interaction Toolkit to automatically segment the bony structures of the mouse from the CT-data using the threshold method to create a 3D-model. Then, this 3D-model of the mouse skeleton was printed using polyactid acid for the bony structures and polyvinyl ether for the support material on a 3D-printer via double-extruding for each (UltiMaker, S5, Utrecht, Netherlands) with the help of the University Center for 3D printing at the University Medical Center Hamburg-Eppendorf. The support material was dissolved in a water bath, so only the bony structures of the mouse skeleton were left. The polyurethane make-up sponge "eggs" were cut in half and glued together to represent the soft tissue of the mouse torso. The skeleton was then glued to the polyurethane mouse torso. We inserted a small polyurethane sponge in the thorax to serve as an attachment point for the suprahepatic vena cava. Finally, the vessels representing the suprahepatic and infrahepatic vena cava were glued to the mouse torso (Fig. 3B-D).

Procedure of orthotopic liver transplantation

Fig. 4 shows the steps performed during orthotopic liver transplantation in live mice. First, donor and recipient mice are anesthetized using isoflurane inhalation and receive buprenorphine as analgesia. At the time of harvest, donor livers are perfused with Perfadex, prior to explantation. Under 30x – 40x magnification, the portal vein and the infrahepatic inferior vena cava are cuffed, and the bile duct is stented. Following abdominal laparotomy in the recipient, hepatectomy is performed. First, the portal vein is cuff-anastomosed, and then the suprahepatic vena cava anastomosis is performed with a 10-0 continuous suture. The infrahepatic anastomosis is carried out using the cuff method. Finally, the bile duct anastomosis is performed by inserting a stent in the simulation donor liver and ligating the stent in place. Thereafter, the other end of the stent is inserted in the recipient's bile duct and is ligated.[11]

Study participants and evaluation

Our study included expert microsurgeons (n = 7) from centers in the US, Canada, Europe, and Asia that had performed more than 100 successful orthotopic liver transplants with a good survival rate in living mice. Participating institutions were in Germany (Hamburg), Canada (Vancouver, Toronto), USA (Pittsburgh, Stanford), Japan (Hiroshima), Belgium (Liege), and Switzerland (Geneva). All selected microsurgeons received a simulation mouse and liver model with corresponding vessels and cuffs. They were asked to perform one orthotopic liver transplantation using the provided models. Afterwards, participants completed a questionnaire with eight evaluation criteria in 3 categories: (1) usability: practicality/simplicity, (2) procedure: time, haptic feedback (for vein and artery), suture behavior, knot tying, and (3) material: rigidity/fragility, wall thickness (both for vein and artery). The participants rated each of these criteria on Likert scales ranging from 1 (negative/different) to 5 (positive/same) for practicability/simplicity, time, haptic feedback, suture behavior, and knot tying. For rigidity/fragility and wall thickness, scales ranging from -2 (too fragile/thin) and +2 (too rigid/thick) were used. Furthermore, they were asked to provide free commentary on the simulation mouse model and the simulation vessels.

Statistics

We used GraphPad Prism 10 (Boston, USA) to compare and visualize the different categories of face validity. Standard error of the mean was calculated.

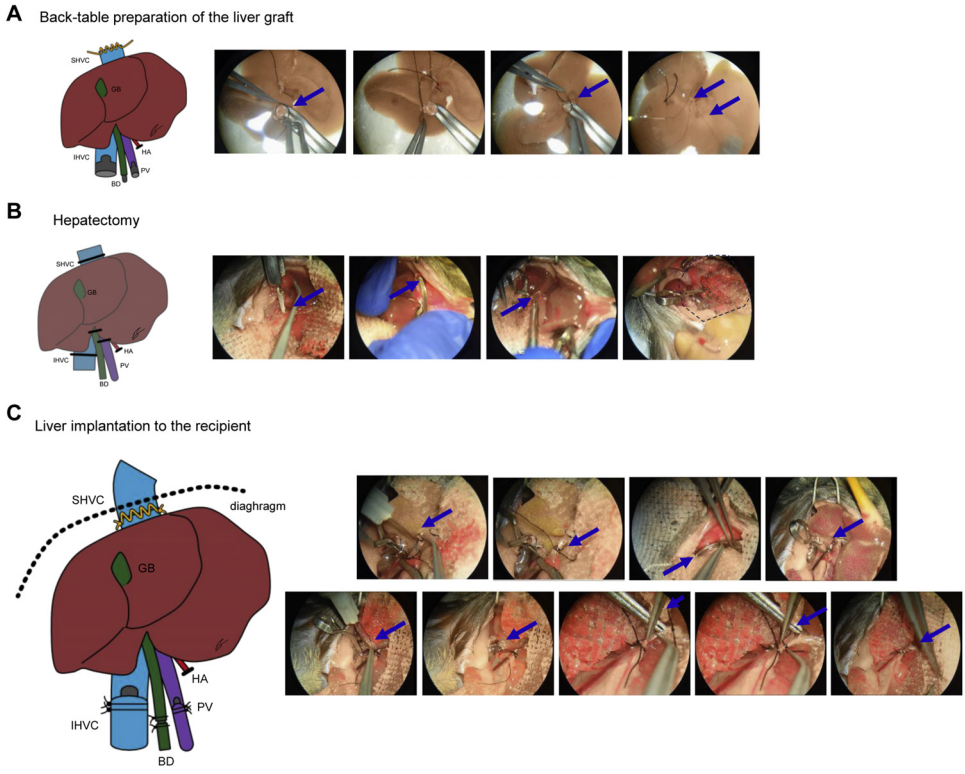


Fig. 4. Murine orthotopic liver transplantation procedure. (A) Back-table preparation of the liver graft with a schematic view of the different anastomoses with a suture anastomosis of the suprahepatic inferior vena cava (SHIVC) and cuff anastomosis of the infrahepatic inferior vena cava (IHIVC), the portal vein (PV), the hepatic artery (HA), and stent anastomosis of the bile duct (BD). First, the IHIVC, PV, and HA are cuffed and ligated, and then a stent is inserted in the BD and ligated. (B) Schematic view of the recipient hepatectomy with transection of the SHIVC, IHIVC, PV, HA, and BD. (C) Implantation of the murine liver starting with the cuff anastomosis of the PV, followed by a suture anastomosis of the SHIVC, and opening of blood flow. Then, the cuff anastomosis of the IHIVC and the HA is performed. Lastly, the BD stent anastomosis is performed.

Ethics statement

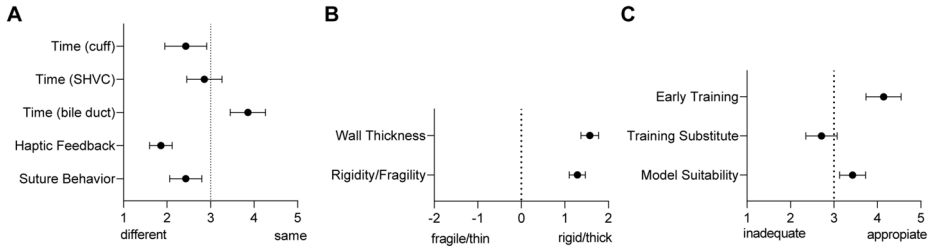
Animal experiments were approved by the local authority (Behörde für Soziales, Familie, Gesundheit und Verbraucherschutz, Hamburg, Germany; N75/2019) and performed in accordance with institutional and European guidelines.

Results

The results of this study were dependent on the expert opinion of surgeons with advanced experience in orthotopic liver transplantation in mice. The overall impression of the surgeons evaluating this model was that it was easy to set up and use. Fig. 5 gives an overview of the answers of the participating surgeons.

Venous anastomosis: Haptic limitations in small, rigid simulation vessels

The participating surgeons reported that the dried latex used was different in its haptic behavior than the wet venous walls of mice. The walls were more rigid and more difficult to evert



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	procedure (1 - 5)								material (-2 - 2)		procedure (1 - 5)		
	1 (different) - 3 (moderate) - 5 (same)								*-2 (too fragile/thin) - 0 (optimal) - 2 (too rigid/thick)*		*1 (different) - 3 (moderate) - 5 (same)*		
rater	**Practicability/Simplicity**	**Time (cuff)**	**Time (SHVC)**	**Time (bile duct)**	**Haptic Feedback**	**Suture Behavior**	**Suture Behavior (Cuff)**	**Knot Tying**	**Rigidity/Fragility**	**Wall Thickness**	**Model Suitability**	**Training Substitute**	**Early Training**
1	5	4	3	5	2	2	4	5	1	2	4	3	5
2	5	1	3	5	3	4	2	3	1	1	3	4	4
3	4	1	1	3	1	1	1	2	2	2	2	1	2
4	4	4	3	4	2	2	4	4	1	2	4	3	5
5	4	2	4	4	1	3	1	3	2	2	3	2	5
6	4	3	4	4	2	3	3	4	1	1	4	3	4
7	4	2	2	2	2	2	4	4	1	1	4	3	4
mean	**4.3**	**2.4**	**2.9**	**3.9**	**1.9**	**2.4**	**2.7**	**3.6**	**1.3**	**1.6**	**3.4**	**2.7**	**4.1**
SEM	*0.24*	*0.68*	*0.49*	*0.37*	*0.37*	*0.51*	*0.68*	*0.51*	*0.24*	*0.2*	*0.37*	*0.51*	*0.58*

Fig. 5. Results of the expert evaluation. Expert surgeons (n=7) from different centers evaluated the model for a total of 10 different categories of face validity (A) Procedure face validity: time, haptic feedback (Suture-, cuff- and stent anastomosis individually), suture behavior, and knot tying were rated on a Likert scale from 1 to 5 (1 – completely different, 2 – rather different, 3 – moderate, 4 – about the same, 5 – the same). Error bars represent SEM. (B) Material face validity: wall thickness and rigidity/fragility were rated on a Likert scale from -2 to 2 (-2 – too fragile/thin, -1 – rather fragile/thin, 0 – optimal, 1 – rather rigid/thick, 2 – too rigid/thick). Error bars represent SEM. (C) Procedure face validity: model suitability and replaceability, and whether it is more suitable for an early training phase were rated on a Likert scale from 1 to 5 (1 – completely different, 2 – rather different, 3 – moderate, 4 – about the same, 5 – the same). Error bars represent SEM. (D) Tabular results by validity category.

once the cuff was inserted. The smaller the vessel diameter, the more marked the difference. Most participating surgeons reported that they needed more time to connect the simulation vessels using the cuff technique than in real mice. The behavior of the vessels in the cuff anastomosis was rated slightly below average compared to the real tissue. Knot tying was rated above average, and thus similar to real mice.

Challenges in anastomosing the suprahepatic vena cava with a continuous suture

The anastomosis of the suprahepatic inferior vena cava was performed using a 10-0 continuous suture. Although the simulation vessel walls were more rigid and thicker, they could be used easily for suturing. The moisture of the vessels in real mice improved handling compared with a dry model. Most evaluators found that they needed more time in the simulation model than in live mice. The suture behavior of the vessels was rated slightly below average compared to the real tissue.

Anastomosis of the bile duct using the stent technique

The anastomosis of the bile duct was only slightly influenced by the rigidity or wall thickness. Most evaluators found this anastomosis to require the same amount of time in the simulation as in live mice.

Evaluation of haptic behavior and knot tying

Most surgeons found that the haptic behavior of the simulation vessels differed from live mice. This may have been due to the fact that rigidity and wall thickness were markedly greater in the simulation models than in live mice, as evident by the respective rating. Knot tying on the simulation vessels was rated above average, close to how it would be in live mice.

Model suitability and training benefits

Overall, the majority of participants found this simulation model appropriate for training orthotopic liver transplantation in mice, although the biggest benefit was replacement of live animal use during the initial training phase. While the model was clearly not intended to replace the complete training process required for surgical proficiency, it effectively mimicked the tight space, constricted movement with each consecutive anastomosis and other challenging factors equivalent to those experienced when carrying out anastomoses in living mice. Simultaneously, this model provided a "clean" working environment, with no connective tissue, no bleeding, and no ischemia time restraints, enabling repeated practice on the same model.

In individual comments, the surgical participants indicated that the model was useful for trainees to build muscle memory, learn suturing of small vessels in a tight space, and memorize the operation sequence (especially for the suprahepatic inferior vena cava anastomosis). The limitations of the model include that it cannot teach the correct handling of delicate wet vessel tissue, especially with regard to cuff anastomosis.

Discussion

The here presented microsurgical simulation model was developed to help meet the demand for training in the basics of orthotopic liver transplantation in mice. It was specifically designed to facilitate the initial stages of learning and dealing with the basic mechanics of liver transplantation in a confined space using different anastomosis techniques. The model allows for realistic vessel suturing and knot tying, although the vessels were evaluated as too rigid and the walls as too thick, making eversion of the vessel walls in the cuff anastomosis technique very difficult. Nonetheless, the model allows for training in the procedure sequence at the back table, as well as in liver implantation. As a dry-lab model, it does not allow for training in dissection and preparation of the recipient mouse. However, this is not the purpose of the model. Our main aim was to create a simple, cost-effective, and easy-to-replicate simulation model that utilizes commercially available materials and allows for training in the procedure without using mice for training purposes, as well as enabling practice outside of a biolab environment. As indicated in a recent systematic review by Franza *et al.*, microsurgical training models can be categorized as non-animal and non-live animal models.[15] With regard to the presented model, it is an intermediate model simulating the confines of the real procedure, but with synthetic materials, thus being able to confer more skill to the trainee than a standard dry-lab model, although not to the extent of a living animal model. In this setting, we believe that it has high potential to reduce animal use for training, especially because it can be easily re-used. This model can also be used in the manufacturing and testing process of prototypes in self-built surgical devices.[16] The main

advantage of this model is the reduction of animal use, especially in the initial learning curve of complex murine abdominal procedures like orthotopic liver transplantation.[11-13] Using the proposed production process, these models could easily be adapted for other animals (e.g., rats) and other complex operations (e.g., lung, kidney, and small intestine transplantation). Training to master the preparation of the donor liver, as well as the implantation procedure using the different anastomosis techniques (running suture, cuff technique, stent technique) in living animals usually produces high casualties, with no direct scientific output. This is not in line with the growing concern regarding the use of animals for teaching and research purposes.[17] When training for the establishment of a new procedure, use of this model may also help secure ethical approval for core animal experiments, due to the total reduction in animal numbers.

This model can be incorporated in a training course to learn the basics of orthotopic liver transplantation, although it cannot replace the use of live animal models completely. There are possibilities to improve this model to overcome the disadvantages of a dry-lab simulation (lack of moisture, connective tissue and very thin and flexible vessel walls, beating organs, blood flow, and bleeding), but this would require considerable effort and would thus defeat the purpose of the simplicity, inexpensiveness, and ease-of-use of this model. It should be discussed when comparing this mouse carcass model with non-living animal vessels (i.e., vessels harvested from e.g., chicken), thus addressing its primary limitation, which is the inflexibility of the vessels. This, of course, counteracts the main aim of not using animals for this simulation model. Another way to further improve the model may simply be to use water or hydrophilic materials, such as konjac flour, for the liver, creating a wet environment that closely resembles that in a living mouse, while retaining simplicity and cost-effectiveness.[18,19] Another possibility is to use polyvinyl alcohol (PVA) hydrogels to recreate soft tissues mimicking the body of the mouse or the liver.[20]

Vascular anastomoses are one of the essential techniques of microsurgery and mastering the exact procedure in murine liver transplantation is key to proficiently perform this procedure. Although the use of live animals is still the “gold standard,”[9] modern microsurgical training consists of a step-up approach with dry-lab and wet-lab training, especially at the beginning of the learning curve, thus minimizing animal use in training.[21,22] Franza *et al.* provide a good overview of non-living models for microsurgical training, using mostly latex or silicone as synthetic materials and chicken, turkey, or pig as non-living materials, with the conclusion that non-living materials can be used in a step-wise approach to develop basic and intermediate skills in microsurgery before transitioning to live animals in accordance with the 3R principles.[15] The advantage of the higher face validity of non-living animal models comes with ethical considerations, cost, and accessibility restrictions. Therefore, dry-lab models such as ours can be used because of their simple and cost-effective nature. Our model can be easily implemented by any lab conducting microsurgical animal procedures, requiring minimal effort and utilizing commercially available latex compounds.

Another interesting aspect is the integration of sensors in this type of simulation models. Such sensors could provide objective performance metrics and real-time feedback for the trainee. Gamberini *et al.* described a sensorized vascular simulator for robotic manipulation, which demonstrated the importance of quantifying tissue strain and differentiating skill levels through construct validity.[23]

Another challenging context in which this model could prove advantageous is in the training of pediatric liver transplantation procedures. Specifically, the small diameter of the vessels and the developing anatomy pose great challenges for the novice surgeon, especially as this procedure can be made even more difficult by the variations in vessel size between donor and the recipient.[24] Due to the technical demands of this procedure, the success of liver transplantation is highly dependent on the skill and experience of the surgeon. Therefore, a dry-lab simulation that facilitates practice in handling vessels with a small diameter would be beneficial in enhancing surgical proficiency in this specialized field.[25]

Limitations of our study include the lack of evaluation of skill improvement (content validity) and the absence of a comparative analysis between expert and novice surgeons (construct validity). It is hard to find reliable information on the learning curve of microsurgeons regarding murine transplantation procedures, so the impact of this model on the learning curve for

murine orthotopic liver transplantation cannot be exactly estimated. It is also difficult to evaluate the costs required per person to acquire this very specialized skill; therefore, the economic impact of such a surgical training model can only be approximately quantified. Our main aim was to evaluate this novel model for feasibility and practical applicability by experts in the field and examine realistic advantages and disadvantages in order to incorporate it within the training modules of aspiring microsurgeons. Future studies implementing the refinements discussed above may allow for a more detailed evaluation of the value of this model.

Conclusion

This model effectively simulates orthotopic liver transplantation in mice, making it an ideal tool for the initial training phase. It is simple, cost-effective, and can reduce reliance on animal use for training. It is effective for procedural training and learning to manage microsurgical skills in a confined space. However, limitations include vessel rigidity and wall thickness, making the cuff technique challenging to master. Potential improvements could involve the development of thinner vessel structures or natural food-like tissue for the liver (eg, Konjac, gelatin), although these modifications might compromise the model's simplicity.

Further analysis of this model should include evaluation of skill improvement (content validity) and a comparative analysis between expert and novice surgeons (construct validity). Overall, this model is a practical and economical option for laboratories and training facilities equipped with microsurgical tools, serving as a low-cost simulator to train essential skills in murine research.

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Declaration of competing interest

The authors of this manuscript have no conflicts of interest to disclose.

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