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Corresponding Author:	Nikolaus Thierfelder Ludwig-Maximilians-Universität München: Ludwig-Maximilians-Universitat Munchen Munich, Bavaria GERMANY
Corresponding Author's Institution:	Ludwig-Maximilians-Universität München: Ludwig-Maximilians-Universitat Munchen
Corresponding Author E-Mail:	Nikolaus.Thierfelder@med.uni-muenchen.de
Order of Authors:	Maximilian Grab
	Carina Hopfner
	Alena Gesenhues
	Fabian König
	Nikolaus Haas
	Christian Hagl
	Adrian Curta
	Nikolaus Thierfelder
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TITLE:

Development and Evaluation of 3D-printed Cardiovascular Phantoms for Interventional Planning and Training

AUTHORS AND AFFILIATIONS:

Maximilian Grab[1,2], Carina Hopfner[3], Alena Gesenhues[4], Fabian König[1,2], Nikolaus A. Haas[3], Christian Hagl[1], Adrian Curta[3], Nikolaus Thierfelder[1]

[1]Department of Cardiac Surgery, Ludwig Maximilian University Munich, Germany
[2]Medical Materials and Implants, Technical University of Munich, Germany
[3]Department Pediatric Cardiology and Pediatric Intensive Care, Ludwig Maximilian University Munich, Germany
[4]Department of Radiology, Ludwig Maximilian University Munich, Germany

Email Addresses of Co-Authors:
Maximilian Grab (maximilian.grab@med.uni-muenchen.de)
Carina Hopfner (Carina.Hopfner@med.uni-muenchen.de)
Alena Gesenhues (alena.gesenhues@med.uni-muenchen.de)
Fabian König (Fabian.koenig@med.uni-muenchen.de)
Nikolaus A. Haas (nikolaus.haas@med.uni-muenchen.de)
Christian Hagl (christian.hagl@med.uni-muenchen.de)
Adrian Curta (adrian.curta@med.uni-muenchen.de)
Nikolaus Thierfelder (nikolaus.thierfelder@med.uni-muenchen.de)

Corresponding Author:
Maximilian Grab (maximilian.grab@med.uni-muenchen.de)

KEYWORDS:

3D-printing, cardiovascular, therapy planning, patient specific, training model, intervention

SUMMARY:

Here we present development of a mock circulation setup for multimodal therapy evaluation, pre-interventional planning, and physician-training on cardiovascular anatomies. With the application of patient-specific tomographic scans, this setup is ideal for therapeutic approaches, training, and education in individualized medicine.

ABSTRACT:

Catheter-based interventions are standard treatment options for cardiovascular pathologies. Therefore, patient-specific models could help training physicians' wire-skills as well as improving planning of interventional procedures. The aim of this study was to develop a manufacturing process of patient specific 3D-printed models for cardiovascular interventions.

To create a 3D-printed elastic phantom, different 3D-printing materials were compared to porcine biological tissues (i.e., aortic tissue) in terms of mechanical characteristics. A fitting

material was selected based on comparative tensile tests and specific material thicknesses were defined. Anonymized contrast-enhanced CT-datasets were collected retrospectively. Patient-specific volumetric models were extracted from these datasets and subsequently 3D-printed. A pulsatile flow loop was constructed to simulate the intraluminal blood flow during interventions. Models' suitability for clinical imaging was assessed by x-ray imaging, CT, 4D-MRI and (Doppler) ultrasonography. Contrast medium was used to enhance visibility in x-ray-based imaging. Different catheterization techniques were applied to evaluate the 3D-printed phantoms in physicians' training as well as for pre-interventional therapy planning.

Printed models showed a high printing resolution (~30 µm) and mechanical properties of the chosen material were comparable to physiological biomechanics. Physical and digital models showed high anatomical accuracy when compared to the underlying radiological dataset. Printed models were suitable for ultrasonic imaging as well as standard x-rays. Doppler ultrasonography and 4D-MRI displayed flow patterns and landmark characteristics (i.e., turbulence, wall shear stress) matching native data. In a catheter-based laboratory setting, patient-specific phantoms were easy to catheterize. Therapy planning and training of interventional procedures on challenging anatomies (e.g., congenital heart disease (CHD)) was possible.

Flexible patient-specific cardiovascular phantoms were 3D-printed, and the application of common clinical imaging techniques was possible. This new process is ideal as a training tool for catheter-based (electrophysiological) interventions and can be used in patient-specific therapy planning.

INTRODUCTION:

Individualized therapies are gaining increasing importance in modern clinical practice. Essentially, they can be classified in two groups: genetic and morphologic approaches. For individualized therapies based on unique personal DNA, either genome sequencing or the quantification of gene expression levels is necessary[1]. One can find these methods in oncology, for example, or in metabolic disorder treatment[2]. The unique morphology (i.e., anatomy) of each individual plays an important role in interventional, surgical, and prosthetic medicine. The development of individualized prostheses and pre-interventional/-operative therapy planning represent central focusses of research groups today[3-5].

Coming from industrial prototype production, 3D-printing is ideal for this field of personalized medicine[6]. 3D-printing is classified as an additive manufacturing method and normally based on a layer-by-layer deposition of material. Nowadays, a broad variety of 3D-printers with different printing techniques is available, enabling processing of polymeric, biologic, or metallic materials. Due to increasing printing speeds as well as the continuous widespread availability of 3D-printers, manufacturing costs are becoming progressively less expensive. Therefore, the use of 3D-printing for pre-interventional planning in daily routines has become economically feasible[7].

The aim of this study was to establish a method for generating patient-specific or disease-specific phantoms, usable in individualized therapy planning in cardiovascular medicine. These phantoms should be compatible with common imaging methods, as well as for different therapeutic

approaches. A further goal was the use of the individualized anatomies as training models for physicians.

PROTOCOL:

Ethical approval was considered by the ethical committee of the Ludwig-Maximilian University and was waived given that the radiological datasets used in this study were retrospectively collected and fully anonymized.

Please refer to the institute's MRI safety guidelines, especially regarding the used LVAD ventricle and metal components of the flow loop.

1. Data acquisition

1.1. Prior to creating the anatomical phantoms, select a suitable radiological dataset, preferably from patients in cardiovascular disciplines. The virtual 3D-model can be derived from both, computed tomography (CT) or magnetic resonance imaging (MRI) datasets.

1.2. Select the pixel size and slice thickness (ST) of the dataset to adapt to the size of the structures intended to be represented in the 3D model. This experiment used an ST of 0.6 mm with a matrix size of 512 x 512 and a field of view of 500 mm leading to a pixel size of 0.98 mm. Ensure that the value of both pixel size and ST must lie below the size of the smallest feature that should be visible in the images and the 3D model, e.g., <0.3 mm for datasets of infants or representation of coronaries, <0.6 mm for the main cardiovascular structures of an adult patient.

1.3. Perform standard acquisition for CT angiography (CTA) in dual-source spiral technique with a ST of 0.6 mm for adult patients. For adults, inject 80 mL of iodine contrast agent at a speed of 4 mL/s and start acquisition 11 s after bolus tracking in the ascending aorta at a threshold of 100 HU. The tube voltage and tube current are selected automatically by the scanner according to the patient's body type. Perform reconstruction in a soft tissue kernel using a high degree of iterative reconstruction.

NOTE: CTA acquisition parameters and protocols are highly dependent on the available CT scanner, patient size and patient circumference. The presented parameters are experience-based and should be taken as a starting point for adjustment rather than a fixed requirement.

1.4. For MR angiography (MRA), perform non contrast enhanced MRA using an in-house modified sequence that utilizes a fully balanced gradient waveform, using both ECG- and respiratory triggering (TE 3.59, TR 407.40, matrix size 224x224). Achieve accelerated MRI data acquisition by using compressed sensing which combines parallel imaging, sparse sampling, and iterative reconstruction. As an example, acquisition times of about 5 min for the thoracic aorta are possible.

1.4.1. Be sure to select a dataset that is free of movement artifacts. To reduce motion artifacts, perform image acquisition using prospective ECG triggering and additional respiratory triggering

for non-CE MRA. Furthermore, when selecting a model for general use, ensure that there are no metallic implants as this can improve the quality of the finished model.

1.5. For the segmentation and 3D-printing of cardiovascular anatomies, use contrast-enhanced datasets. The use of native cardiovascular datasets makes the separation of hollow anatomic structures (e.g., vessels or ventricle) from blood difficult, due to comparable Hounsfield values of roughly 30 HU[8].

NOTE: A higher Hounsfield value gradient between blood volume and surrounding soft tissue will allow for an easier separation in the segmentation process. When the gradient is very small, parts of the soft tissue will be displayed as part of the blood volume, resulting in a poor model quality and additional post processing.

1.6. When exporting the dataset, make sure to select a reasonably low slice thickness (roughly 0.3 - 0.6 mm for CTA and 0.8 – 1.0 mm for MRA), since the resolution and surface quality of the printed model depends greatly upon this parameter.

NOTE: If the slice thickness is too thin, the required computing power for modeling will increase substantially, which slows the process accordingly. On the other hand, excessive slice thickness can result in the loss of small details in the patients' anatomy.

2. 3D-model creation

NOTE: The creation of a 3D-model from a radiological dataset is called the segmentation process, and a special software is required. The Segmentation of medical images bases itself upon Hounsfield units, to form 3-dimensional models[9]. This study uses a commercial segmentation and 3D-modeling software (see **Table of Materials**), but similar results can be achieved using available freeware. The following steps will be described for modeling from a contrast-enhanced CT dataset.

2.1. After importing the dataset into the segmentation software, crop the dataset to limit the area of interest, i.e., heart and aortic arch. Achieved this by selecting the **Crop Images** tool and moving the edges of the ROI by clicking and moving the sides of the frame. This can be done in all three orientations. Therefore, a focus on the ROI, together with a decrease of file size is obtained, which enables higher computing speed, leading to reduced overall working time.

2.2. Define a range of Hounsfield unit values (approx. 200-800 HU) by opening the **Thresholding** tool, resulting in a combined mask of the contrast-enhanced blood volume and bone structures (**Figure 1A**, e.g., sternum, parts of the ribcage, and spine).

2.3. Remove all bone parts which are undesirable in the final 3D-model by using the **Split Mask** tool which enables the marking and separation of multiple areas and overall slices, based on Hounsfield values and location.

2.4. Following this separation, ensure that a mask containing the contrast-enhanced blood volume remains. This can be done, by scrolling through the coronal and axial planes and matching the created mask with the underlying dataset. From this mask, calculate a rendered 3D polygon surface-model (the so-called STL) (**Figure 1B**).

NOTE: Tool names might differ in other segmentation programs.

2.5. For further adaption and manipulation, transfer the 3D-model to a 3D-modeling software (see **Table of Materials**). To export the 3D-model, click on the **Export**-Tool and select the 3D-modeling software, or a fitting data format for the exported file. Subsequently, confirm your selection and the export process will be performed.

2.6. Use the **Trim** tool to crop the blood volume to the specific area of interest (e.g., removing parts of the aorta or some of the heart cavities). Click the tool and draw a contour around the parts that needs removal.

NOTE: Depending on the dataset quality and the accuracy of the segmentation, some minor surface repairs and modifications might be required at this point. Further design operations allow the manipulation of patient-specific models according to the purpose of use, e.g., in training. Some examples for engineering, according to the patients' anatomy, include scaling the entire model or single structures, to create or delete connections, combining parts of different models in one. Such features are particularly interesting for training models with congenital abnormalities, as CT and MRI images are rare in pediatrics, where the minimization of radiation and sedation is key. Therefore, the adaptation and modification of existing models is especially helpful for the 3D printing of congenital heart defect models.

2.7. Click the **Local Smoothing** tool to adjust the surface of the segmented model manually and locally. Focus on removing rough polygon shapes, single peaks and rough edges created by the previous trimming operations.

2.8. To allow the later connection of the model to a flow loop, include tubular parts with defined diameters adjusted to the available hose connectors and tube diameters (**Figure 1C**). Therefore, place a datum plane parallel to the opening cross-section of the vessels at a distance of roughly 10 mm.

2.8.1. To place the plane, select the tool **Create datum plane** and use the preset **3-point plane**. Next, click on three equally spaced points on the vessels cross-section to create the plane. Afterwards, input an offset of 10 mm in the command window and confirm the operation.

2.8.2. Select the **Draw Sketch** tool from the menu and choose the previously created datum plane as location of the sketch. In the sketch, place a circle roughly on the centerline of the vessel and set the radius constraint to match the outer diameter of your hose connector (24 mm for aortic inlet, 8-10 mm for subclavian, carotid, and renal vessels, and 16-20 mm for the distal opening of the vessel).

2.9. From the created sketch, use the **Extrude** tool to create a cylinder with a length of 10 mm. Orient the extrusion to move away from the vessel opening, to create a distance between the cylinder and the vessel cross-section of 10 mm. Then, use the **Loft** tool, to create a connection between the vessel ending and the geometrically defined cylinder is established. At this point, ensure a smooth transition between the two cross-sections, thereby avoiding turbulence and low flow areas in the final 3D flow model (**Figure 1D**).

NOTE: By following these steps, a 3D-model of the blood volume of the aorta and adherent arteries will be created. Furthermore, it will include the connectors required for subsequently connecting it to a flow loop.

2.10. To make a hollow blood space, use **Hollow** tool in the software. In the command window, input the required wall thickness (in this experiment: 2.5 mm) Furthermore, the direction of the hollowing process has to be set to "move outward". Afterwards, confirm selection and the hollowing process will be executed.

NOTE: This step allows the selection of a fixed wall thickness for the entire model. Since "hollowing" creates a defined wall thickness on all surfaces, a fully closed model will result. Therefore, the ends of all vessels will need to be trimmed once more using the step described in step 2.6 (**Figure 1E**). When using flexible 3D-printing materials, this step is essential to define the final bio-mechanic properties of the phantom. By increasing the wall thickness of the model, higher resilience and lower elasticity will logically result. If the mechanical properties of the native tissue and the 3D-printing material are not known, tensile tests must be performed at this point. Since the wall thickness is constant across the entire model, the desired mechanical properties should be recreated in the region of interest of the model.

2.11. Some processing software offer a "Wizard" to ensure the printability of the final model, which is highly recommended. This optional processing step will analyze the models' polygon mesh and mark overlaps, defects and small objects, which are not connected to the model. Usually, the wizard offers solutions to remove the found issues, resulting in a printable 3D-model (**Figure 1F**).

2.12. Export the final model as .stl-file by selecting the **Export** option in the **File** tab.

NOTE: To confirm the accuracy of the designed 3D-model, some software enables the overlay of the final STL's contour and the underlying radiological dataset. This allows a visual comparison of the 3D-model to the native anatomy. Furthermore, a printer with a suitable spatial resolution of < 40 µm must be selected, to allow for an accurate print of the digital model.

3. 3D-printing and flow loop setup

3.1. Upload the .stl-file to a 3D-printer, using the slicing software provided by the manufacturer, to produce a physical phantom of the anatomy. Ideally, one should use a printing layer height of ≤ 0.15 mm to ensure high resolution and good printing quality.

NOTE: There is a wide range of elastic printing materials and suitable 3D-printers available on the market. Different setups can be used to print the previously described digital models. However, resolution, post-processing and mechanical behavior might differ from the presented results.

3.2. After uploading the printing file from the slicing software to the 3D-printer, ensure that the amount of printing material and support material in the printer's cartridges is sufficient for the 3D-model and start the print.

3.3. Following the printing process, remove the support material from the finished model. First, remove the support material manually by gently squeezing the model, followed by immersion in water or a respective solvent (depending on support material). Dry in an incubator set to 40 °C overnight.

NOTE: The removal of the support material can be a time-consuming step, depending on the complexity of the anatomical model. While the use of tools like spatulas, spoons and medical probes can slightly decrease the post processing time, it also increases the danger of perforating the model's wall, rendering it useless for fluid testing. When using the Polyjet printing technology, the entire model will be encased by a support material. This is required to keep the uncured model material in place while it is cured using UV-light. In hollow tubular models, this will lead to a much higher demand for support material compared to actual model material. The model presented in **Figure 2** uses roughly 200 g of model material and 2,000 g of support material.

3.4. Next, embed the model in 1% agar. This reduces movement artifacts during clinical imaging of the model. Secondly, agar offers a better haptic feedback during sonographic imaging, and a better force feedback during catheterization, as compared to submersion in water.

3.4.1. Use a plastic box with at least 2 cm side margins around the model. Drill holes into the walls of the box to allow the tubes to be connected from the vessels to the pump and the reservoir.

3.4.2. Prepare an agar solution by adding 1% w/v in water and bringing to a boil. After boiling and stirring the mixture, let it cool for 5 min and pour into the box to create a bed of at least 2 cm height, on which the model will be placed.

NOTE: If the model is placed directly onto the bottom of the box, the pulsatility of the fluid inside the model will create an asymmetric upward movement.

3.5. While the agar bed sets, connect the model to non-compliant PVC tube, using commercial hose connectors at every opening. A tube diameter of 3/8" is recommended for large vessels

(e.g., aorta) and/or anatomical structures with high blood flow (e.g., ventricles). For smaller vessels a 1/8" tube is sufficient. Use zip ties to fix the connection between the hose connectors and the 3D-model and ensure there is no fluid leakage.

3.6. Guide the PVC tubes through the drilled holes into the box and then place the model on top of the set agar bed. To prevent agar leaking from these holes, use heat proof modeling clay to seal it. Subsequently, fill the box with agar, covering the model by adding a 2 cm layer on top and leaving for an hour at room temperature for the agar to fully cool and set. This will require more of the agar mixture described in step 3.3.

NOTE: The agar once cured will be usable for about a week, if refrigerated. Once it visibly reduces in volume, it should be replaced by a fresh batch.

3.7. Connect a pulsating pneumatic ventricle pump to the model using the 3/8" tubing attached to the proximal opening. Connect the other tubes to the reservoir and subsequently, connect the reservoir to the inlet of the ventricle pump to create a closed flow loop. (**Figure 2**; e.g., ventricular assist device (VAD)-ventricle). The pump should have a stroke volume of 80 – 100 mL to ensure sufficient physiological flow in adult anatomies. For pediatric anatomies, smaller pumping chambers are available.

3.8. Agitate the ventricle using a piston pump with a stroke volume of 120 – 150 mL, to account for air compression in the connective tube system.

4. Clinical imaging

NOTE: To prevent artifacts in clinical imaging, it has to be ensured that there are no air pockets in the fluid circuit.

4.1. CT imaging

4.1.1 For CT imaging, place the entire flow loop within the CT scanner with the drive unit standing close by. Connect the contrast agent pump directly to the reservoir of the flow loop, so the flooding of the model with contrast agent can be simulated during scanning. This is especially useful for visualizing vascular pathologies.

4.1.2 Perform CT as a dynamic scan over the whole model to visualize contrast agent inflow. Tube voltage is set at 100 kVp, tube current at 400 mAs. Collimation is 1.2 mm. Inject 100 mL of 1:10 diluted iodinated contrast agent into the model's reservoir, at a speed of 4 mL/s. Start the scan using bolus triggering in the leading tube, with a 100 HU threshold and 4 s delay.

4.2. Sonography

4.2.1. Put a small amount of ultrasonic gel on top of the agar block to reduce artifacts. Start the pump and use the ultrasonic head to locate the anatomical structure of interest for ultrasonic

imaging (i.e., heart valves). Use 2D-echo mode to evaluate leaflet movement, as well as opening and closing behavior of the valve. Use color Doppler to evaluate blood flow across the valve and spectral Doppler to quantify the flow velocity following the heart valve.

4.3. Catheterization/ interventions

4.3.1. Insert an access port into the PVC tube directly below the 3D-model, to allow for an easier access of the anatomy with a cardiac catheter or guidewire. After starting the flow loop, check for leakage at the port entrance point. If necessary, use a two-component adhesive to seal the opening.

4.3.2. Place the 3D model on the patient table underneath the C-arm(s) of the X-ray machine. Use X-ray imaging to guide the catheter and guidewires through the anatomic structure. For balloon dilation or stentgraft placement use continuous X-ray mode to visualize the expansion of the device.

NOTE: Catheterization and intervention training on 3D-printed models allows for the interchangeable use of different anatomical and pathological models. This further increases the variety and realism of the training setting.

4.4. 4D-MRI

4.4.1. Use a 1.5 T scanner for MRI acquisition and ensure that the acquisition protocol consists of a non-contrast-enhanced MRA as described above and the 4D-Flow sequence. For 4D-Flow acquire an isotropic dataset with 25 phases and a slice thickness of 1.2 mm (TE 2.300, TR 38.800, FA 7 °, matrix size 298 x 298). Set the velocity encoding at 100 cm/s. The in vitro measurements are performed using simulated ECG- and respiratory triggers.

4.4.2. For 4D-Flow analysis the box with the embedded model and the VAD-ventricle are placed in the MRI scanner and covered with an 18-channel body coil. With regard to the magnetic field of the MRI scanner, the pneumatic drive unit has to be placed outside the scanner room; therefore, a longer connective tube system is usually required.

4.4.3. Perform the 4D-Flow image analysis with a commercially available software. First, import the 4D-MRI dataset by selecting it from the flash drive. Next, perform semi-automated offset correction and correction of aliasing to improve image quality. Subsequently, the centreline of the vessel is automatically traced, and the software extracts the 3D volume.

4.4.4. Finally, perform quantitative analysis of flow parameters by clicking on the individual tabs in the analysis window. Flow visualization, pathline visualization, and flow vector will be visualized without further input. For quantification of pressure and wall shear stress in the respective tab, place two planes by clicking on the button **Add Plane**. The planes will be automatically placed perpendicular to the vessels' centreline.

4.4.5. Move the planes to the ROI by dragging it along the centreline, so one plane is placed at the beginning of the ROI and one at the end. In the diagram next to the 3D-model the pressure drop across the ROI and wall shear stress will be visualized and quantified.

REPRESENTATIVE RESULTS:

The described representative results focus on a few cardiovascular structures commonly used in planning, training, or testing setting. These were created using isotropic CT-datasets with a ST of 1 mm and a voxel size of 1 mm^3. The aortic aneurysm models' wall thickness was set at 2.5 mm complying with comparative tensile testing results of the printing material (tensile strength: 0.62 ± 0.01 N/mm^2; F_{max}: 1. 55 ± 0.02 N; elongation: 9.01 ± 0.34 %) and porcine aortic samples (Width: 1 mm; F_{max}: 1.62 ± 0.83 N; elongation: 9.04 ± 2.76 %).

The presented 3D-printed models offer a wide range of possibilities in CT-imaging. The printed material can easily be distinguished from the surrounding agar and possible metallic implants (**Figure 3A**). Therefore, the use of a contrast agent is normally not required, except for generating dynamic imaging sequences. This can be especially useful for the evaluation of endovascular stentgrafts, since it allows for the visualization of possible prosthesis mismatches and subsequently appearing endoleaks.

As a staple in daily clinical work, sonographic imaging is a prime example for the application of 3D-printed models as training setups. It can be used for both the evaluation of heart valve dynamics, as well as investigation of the whole heart, particularly in pediatrics. Ultrasonic imaging of the 3D-printed model reveals a good permeability of the ultrasonic waves. Furthermore, it is possible to distinguish between the model's wall, the surrounding agar and thin dynamic objects, like heart valve leaflets (**Figure 3B**). The agar layer on top of the model provides realistic haptic feedback during the scanning process.

The usage of 4D-MRI in the flow analysis within the flow loop offers a wide range of possible applications in pre-interventional imaging. 4D-MRI sequence enables visualization of fluid flow, turbulences, and wall shear stress within the 3D-printed model. This allows for the analysis of flow patterns following artificial heart valves, which can lead to high wall shear stress and turbulence in the ascending aorta and aortic arch (**Figure 3C**). The impact of turbulence and high wall shear stress is specifically interesting for the analysis of aortic aneurysms. Thus, the 3D-models can help to better understand the occurrence of aneurysms in both the thoracic and abdominal aorta.

3D-printed cardiovascular models provide a realistic training environment for diagnostic and interventional cardiology. The simulation setup allows the trainees to practice the handling of guiding wires/catheters and maneuvering through the vessels and heart structures, intracardiac pressure measurements, balloon dilatation of stenotic vessels or valves, positioning and dilation of stents, as well as angiographic imaging (visualization of inner structures of the 3D model, e.g., heart valves). The skills and tasks for both roles, first and second operator, as well as the communication amongst to two are included during the training. Modification of the 3D printed models in the 3D-modeling software enables the adaptation of the model structure and size

(infant to adult) to any training level and goals. Therefore, students as well as proficient practitioners benefit from the training to the same extent. Workshops for all training levels – medical students to pediatric cardiologists with years of experience - have successfully been carried out on 3D models representing the most common congenital defects, which include patent ductus arteriosus (PDA), pulmonary valve stenosis (PS), aortic valve stenosis (AS), coarctation of the aorta (CoA) and atrial septal defect (ASD). The appearance of the 3D models under X-ray imaging, as well as the haptic feedback from the manipulation of the instruments inside the model, were assessed as extremely realistic. Repetitive training on 3D models leads to well-versed orientation in 3D, improved perception of haptic feedback and – most important for the patient - minimization of radiation exposure.

FIGURE AND TABLE LEGENDS:

Figure 1: Design steps from a radiological dataset to a printed anatomical model (Pathology: infrarenal aortic aneurysm). (**A**) CT-dataset-based segmentation process (**B**) Rough 3D-model after segmentation (**C**) Smoothed model with added tubular connectors (**D**) Final model of the blood volume with connectors (**E**) Hollow model with defined wall thickness (**F**) 3D-printed flexible model.

Figure 2: Setup of the flow loop. (**A**) Schematic model of the flow loop (**B**) Final flow loop setup with LVAD (1), embedded model (2), a reservoir (3) and a 3D-printed tube connector (optional) (4)

Figure 3: Clinical imaging techniques. (**A**) CT-reconstruction of an aortic arch with a biological surgical heart valve (**B**) Ultrasonic image of a 3D-printed aortic root (1) with an open biological surgical heart valve (2) (**C**) 4D-MRI flow visualization in the aortic arch (**D**) X-ray imaging of a 3D-printed pediatric heart (1) during a catheter intervention (2)

DISCUSSION:

The presented workflow allows to establish individualized models and thereby perform pre-interventional therapy planning, as well as physician training on individualized anatomies. To achieve this, patient-specific tomographic data can be used for segmentation and 3D-printing of flexible cardiovascular phantoms. By implementation of these 3D-printed models in a mock circulation, different clinical situations can be realistically simulated.

Nowadays, many therapy planning procedures focus upon the digital simulation of different scenarios, in order to identify the most favorable outcome[10,11]. In contrast to these in-silico simulations, the described 3D-printed setup enables tactile feedback in training procedures; a material compliance close to the human original is possible in pulsatile perfusion. On the other hand, many published 3D-printed cardiovascular phantoms only use rigid material and therefore are limited to a mainly visual use[12,13].

However, it must be understood that current 3D-printing techniques and materials remain the biggest limitation in reproducing biomechanical properties for the presented workflow[14]. While

an exact recreation of the anatomical shape is possible, the mechanical behavior of the created models will still differ from native aortic tissue to an extent. To mimic different tissues with varying bio-mechanical properties in one phantom, so far as it is possible at all, can be accomplished only by a few sophisticated multi-material 3D-printers[15]. Creating tissue mimicking materials for 3D-printing remains a focus of scientific research; the development of novel materials will result in even more realistic results[16,17]. As long as, only commercially available printing material and/or one-component-printing is available, the mechanical properties of the phantom can be adjusted by means of variations of the wall thicknesses, as was conducted in this study. It is, therefore, not recommended only to duplicate the thickness of the tissue of interest from the underlying tomographic data. It is important to stress that there exists a wide range of different 3D-printers with different materials and varying mechanical properties on the market[18]. Comprehensive mechanical testing is, therefore, recommended, prior to 3D-printing. For printing of cardiovascular structures, (i.e., aortic or ventricular walls), different native tissue samples are required for reference. Following the described segmentation and printing workflow, the creation of flexible and anatomically accurate as well as engineered but realistic 3D-printed models of a wide range of cardiovascular anatomies is possible.

The cost-effectiveness of 3D-printed models depends significantly upon the material properties. In interventional training the high durability of each model (even after balloon dilation) is necessary, in to reduce overall costs. When looking at patient-specific therapy planning, one must take into account the beneficial effect of a printed model. A 3D-printed model will not prove cost-effective for a “standard” surgical patient, but might offer tremendous insight in patients with complex anatomies. Therefore, the costs of training models have to be weighed against their prospective benefits.

Until now, a few commercially available phantoms for clinical training exist on the market; some academic models have been published[19,20]. These models normally have pre-defined anatomies and usually prove difficult to employ in patient-specific settings. Furthermore, high acquisition costs complicate the widespread use of these tools in physicians’ training. The presented customizable mock circulation can be created on a low budget if necessary. Tomographic, fluoroscopy and sonographic scanners, for acquisition of the patient-specific data as well as for the later use of the mock circulation, are standard equipment of any general or university hospital in developed countries. Segmentation of the cardiovascular anatomy and creation of the virtual 3D-model can be performed with the mentioned licensed software, but freeware is also available[21]. The freeware options offer excellent results when creating 3D-models from radiological datasets, although a high amount of initial work is required to adjust the software to individual needs. Furthermore, a subsequent editing of the digital 3D model requires an additional software, which is why a comprehensive software suite covering all these aspects is highly recommended for a quick and smooth workflow. If necessary, printing of the flexible phantoms can be done by contract 3D-manufacturing if there is no suitable 3D-printer on site. By anatomical reduction on the region of interest, the size of the 3D-printed phantom can be reduced, which comes with faster printing times and lower costs.

The most critical point of the process described above is the initial image acquisition. As a result,

the higher the quality of the tomographic data, the more accurate will prove the final 3D-printed phantom. There are two major factors in obtaining suitable data from CT or MRI: Prevention of artifacts and spatial resolution. To prevent artifacts, ideally no metallic materials (e.g., implants) will be next to the region of interest, if no specific artifact reduction techniques are available[22]. In order to reduce motion artifacts, ECG- and respiratory triggering should be performed during image acquisition[23,24]. Spatial resolution depends on the imaging device; however, a slice thickness of 1.0 mm or less is necessary to obtain suitable 3D-printed phantoms without excessive digital postprocessing.

The above-mentioned modularity, cost effectiveness as well as versatility predisposes the individualizable mock circulation for supplementary use in daily clinical routine. The presented method can be beneficial for a wide range of clinical and basic research fields. The use of realistic models is excellent for teaching young doctors and students the basics of sonography, as well as interventional techniques. Especially with interventions, such a model will render the technology more accessible and increase the overall knowledge base of doctors, long-term. CT and MRI imaging, especially when looking at hemodynamic flow patterns in the aortic vessels, can be a major addition both in basic science, as well as determining the outcome of surgical and transcatheter interventions.

ACKNOWLEDGMENTS:
This publication was supported by the German Heart Foundation/German Foundation of Heart Research.

DISCLOSURES:
The authors declare no conflict of interest.

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Figure 1

Click here to access/download;Figure;Fig_1.png

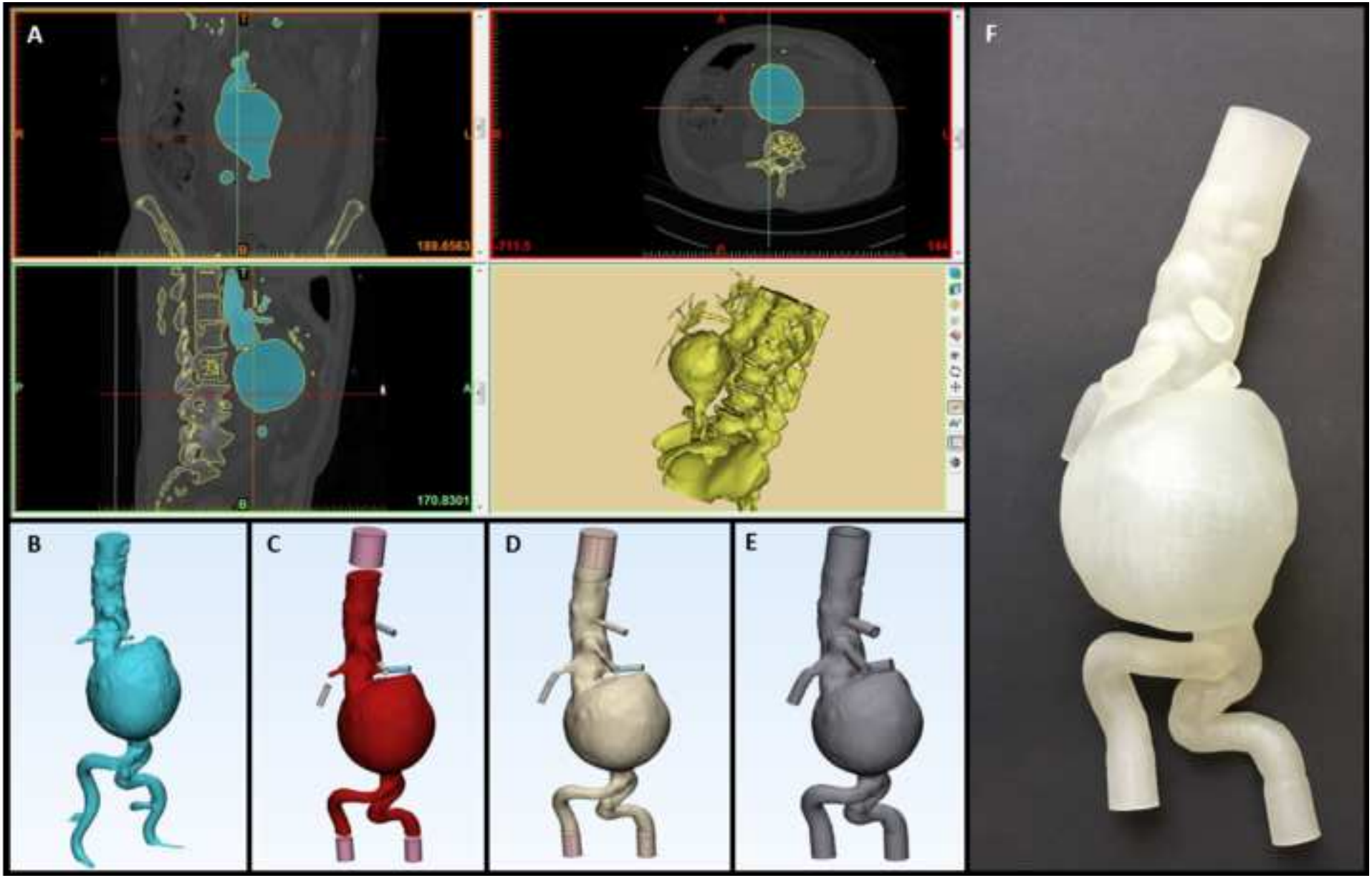


Figure 2

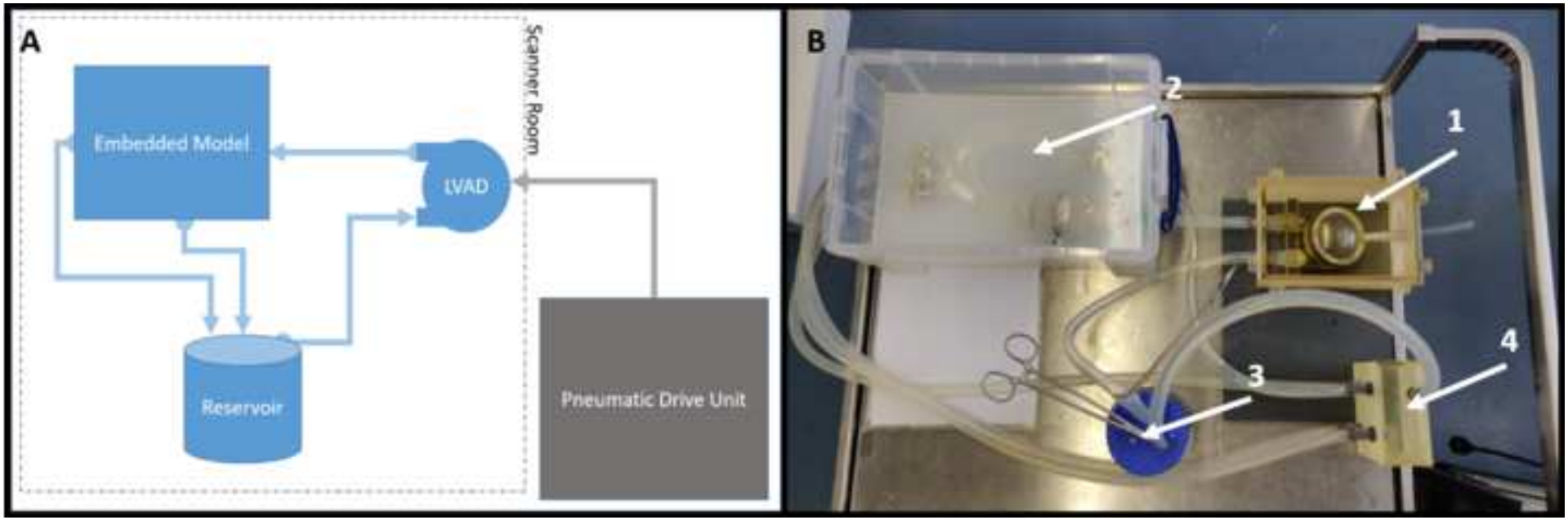
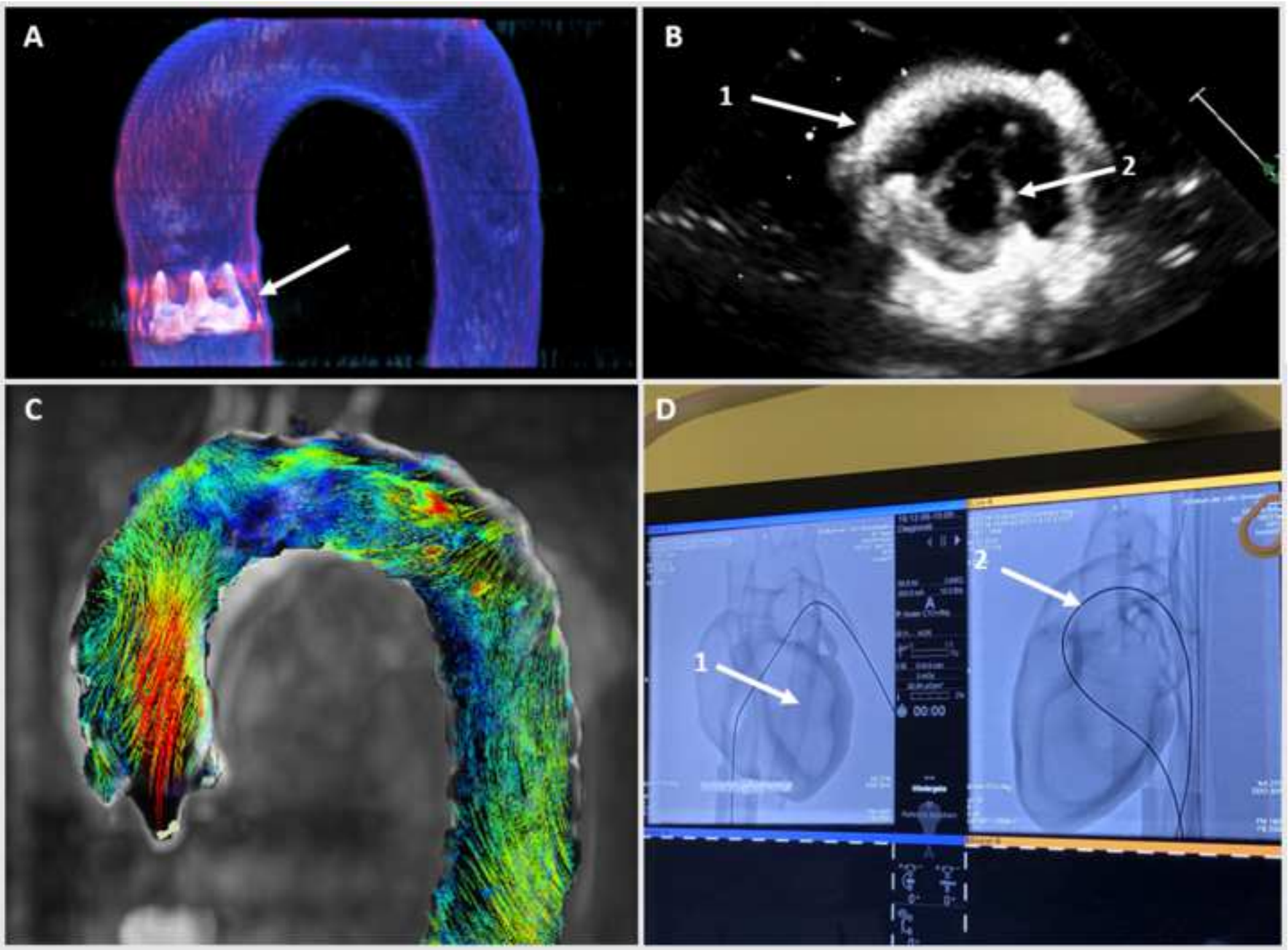


Figure 3

Click here to access/download;Figure;Fig_3.png



3D printable model of thoracic aortic aneurysm

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Video or Animated Figure

3D_model.stl

Name of Material/ Equipment	Company	Catalog Number
3-matic	Materialise AB	
Affiniti 50	Philips Medical Systems GmbH	
Agilista W3200	Keyence Co.	
AR-G1L	Keyence Co.	
Artis Zee	Siemens Healthcare GmbH	
cvi42	CCI Inc.	
Diagnostic Catheter, Multipurpose		
MPA 2	Cordis, A Cardinal Health company	
Excor Ventricular Assist Device	Berlin Heart GmbH	
Imeron 400 Contrast Agent	Bracco Imaging	
IntroGuide F	Angiokard Medizintechnik GmbH	
Lunderquist Guidewire	Cook Medical Inc.	
MAGNETOM Aera	Siemens Healthcare GmbH	
Magnevist Contrast Agent	Bayer Vital GmbH	
Mimics	Materialise AB	
Modeling Studio	Keyence Co.	
PVC tubing		
Radifocus Guide Wire M	Terumo Europe NV	
Really useful box 9L	Really useful products Ltd.	
Rotigarose - Standard Agar	Carl Roth GmbH	3810.4
Solidworks	Dassault Systemes SE	
SOMATOM Force	Siemens Healthcare GmbH	
syngo via	Siemens Healthcare GmbH	

Comments/Description

Software Version 15.0 - Commercial 3D-Modeling Software
Ultrasonic Imaging System
Polyjet 3D-Printer with a spatial resolution of 30µm
flexible 3D-Printing material
Angiographic X-ray Scanner
Software Version 5.12 - 4D Flow Analysis Software

Catheter for pediatric training models, Sizes 4F for infants and 5F for children, young adults
80 -100ml stroke volume
CT - Contrast Agent
Guidewire with J-tip; diameter: 0.035" length: 220cm
(T)EVAR interventional guidewire
MRI Scanner
MRI - Contrast Agent
Software Version 23.0 - Commercial Segmentation Software
3D-Printer Slicing Software

Straight guidewire; diameter: 0.035" length: 260cm

Software Version 2019-2020; CAD Design Software
Computed Tomography Scanner
Radiological Imaging Software

Review Responses

Editorial Comments

We thank the editor for assessing the manuscript and giving valuable input on how to improve the overall manuscript and comply with publishing guidelines

1. *Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please define all abbreviations at first use.*

   Authors: The authors excuse for spelling or grammar issues. The manuscript was carefully revised in order to prevent language mistakes.

2. *Please remove headings from the abstract (objectives, methods, etc).*

   Authors*:* The authors thank the editor for highlighting this aspect. We adjusted the abstract and removed all headings from the individual passages (p: 2, l: 40, 45, 55, 64)

3. *Please revise the following lines to avoid overlap with previously published work: 45-52,55-58.*

   Authors: We thank the editor for highlighting this oversight and we apologize for the overlap with a previously published abstract from a conference. Page 2 lines 44-50 and 54-59 were adjusted.

4. *JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.*

   For example: Materialise NV, Leuven, Belgium; Materialise Mimics 23.0; Materialise 3-matic 15.0; Berlin Heart Excor; CV142, Circle Cardiovascular, Calgary, CA;

   Authors: All paragraphs containing company or product names were adjusted to use a broader description of the respective tool. Concrete company and product names, as well as software versions were added to the table of materials.

5. *Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).*

   Authors: The authors excuse for this stylistic mistake. All passages using personal pronouns were revised and adjusted accordingly.

6. *Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.*

   Authors: We thank the editor for highlighting the need to provide a more imperative writing style in the methods section. Therefore, the authors edited single steps in the Methods section

that should be understood as a command rather than additional information, or possible use cases.

7. *Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. The video should contain a representative (highlighted) excerpt of your protocol with enough specific details to allow readers to execute your protocol and modify it for their own purpose.*

   Authors*:* The authors agree that a precise description, especially of single software steps is necessary to ensure a reproducibility based on the presented method. Therefore, two untrained students tried to follow the presented process in order to create a digital cardiovascular model from a CT-dataset. Based on their feedback we updated several steps to make the use of individual tools easier and clearer. We hope these changes are satisfactory for the editor.

8. *As we are a methods journal, please add to the Discussion modifications and troubleshooting and limitations of the technique.*

   Authors: The authors thank the reviewer for highlighting this point. The discussion was carefully revised and modified in order to comply with this requirement. (e.g.: p. 11, l. 391-393; p. 11, 398-401 or p. 12, 403-409)

9. *I Please include an Acknowledgements section, containing any acknowledgments and all funding sources for this work.*

   Authors: The authors excuse that the Acknowledgement section was missing in the manuscript. This part was updated and now includes relevant funding.

10. *J Please do not abbreviate journal names in the reference list.*

    Authors: The reference list was adjusted to include the full journal names instead of abbreviations.

11. *Please sort the Materials Table alphabetically by the name of the material.*

    Authors: Subcategories were removed and the materials table was sorted alphabetically.

Reviewer 1 Comments

Authors: The authors thank the reviewer for his comments and highly valuable input on the underlying topic of tissue mimicking 3D-printable materials. While we addressed all comments separately, we want to address the ambiguous parts of the presented manuscript with regards to the mentioned materials. Since the journal guidelines explicitly prohibit the use of commercial product names, we generally tried to comply with this rule. At certain points, we deemed it necessary to clearly state the material used, due to its high impact on the presented method. This created some unwanted

incoherence, which is why we removed all specific materials completely from the manuscript. We added some additional information for the different comments below and hope to have answered your questions sufficiently. All materials used in this study can be found in the table of materials.

Major Concern: The results in the paper use commercially available software (cvi42 etc) and printers (Polyjet), lack novelty and originality. Meanwhile, no mechanical results and analytical discussion about the material's property provided, even the author mentions it is similar to tissue, without evidence, it can only be defined as flexible material instead of tissue-mimicking material. The 4D flow analysis seems irrelevant to the X-ray imaging results, should be relevant to MRI imaging results.

Authors: The authors agree with the reviewer's statement, that tissue-like properties can't be claimed without the underlying mechanical data of the printed phantoms. The main focus of this manuscript is the methodological description of the design/manufacturing process, used by our research group to create anatomical 3D-printed models that are used in a wide array of applications ranging from testing to planning and teaching. Therefore, we tried to resolve your concern by adding additional tensile testing data, we performed to compare our printing material to native porcine aortae (Non-pathologic human reference material has an extremely restricted availability). While this doesn't offer a comprehensive analysis of printing material, especially when compared to the publications mentioned in the reviewer's comment no. 5, the authors strongly believe that this manuscript offers a great benefit to research groups trying to replicate the success we see with 3D-printed models in our research and clinical work. Further improvements can and should be made to the printing material, especially with the ongoing rapid development of new printing materials and multimaterial printer technologies.

The authors agree, that the 4D-flow analysis has only limited comparability to the underlying x-ray imaging. The results described are meant to be a representative showcase of the possibilities of the design workflow in combination with radiological imaging. Since most cardiothoracic patients undergo a pre-procedural CT-scan, it is the most realistic situation to create a 3D-model from a patient's CT-data. A mandatory 4D flow analysis of cardiovascular patients is still a few steps away, due to accessibility and licensing issues. Nevertheless, it will offer a lot of clinical benefits, especially for follow-up assessment.

Minor Concerns: The author keeps missing the keywords and using ambiguous description throughout the paper, better to be more specific. The detailed comments are listed below:

Authors*:* Since the journal guidelines prohibit the use of commercial language in the manuscript, the authors deemed it important to add the commercial names at spots where the specific material is important or mandatory (i.e. cvi42 analysis software). The authors acknowledge that this led to an unspecific description pattern. Therefore, to comply with journal guidelines, we removed all commercial language from the manuscript. We hope that the updated table of materials gives the reviewer a better insight into the materials used.

1. *Line 45-46: Different flexible 3D-printing materials were compared to biological tissues in terms of mechanical properties.*

   Comments: It is worth mentioning what the flexible 3D printing materials are, for example, agar, silicone, etc. Meanwhile, in the following sections, the comparison results also needs to be demonstrated to prove the final chosen material is an appropriate material, not just simply define it is an appropriate material, for example, using Young's modulus and Shore A hardness results.

   Authors: We thank the reviewer for this valuable comment. The material used for the printed models is Keyence AR-G1L, an elastic silicone-like material with a Shore A hardness of 35.

Tensile testing results were added to the representative results section: "*The aortic aneurysm models' wall thickness was set at 2.5 mm complying with comparative tensile testing results of the printing material (tensile strength: 0.62 ± 0.01 N/mm²; F_{max}: 1. 55 ± 0.02 N; elongation: 9.01 ± 0.34 %) and porcine aortic samples (Width: 1 mm; F_{max}: 1.62 ± 0.83 N; elongation: 9.04 ± 2.76 %). For printing other cardiovascular structures, (i.e. ventricular walls), different native tissue samples are required for reference.*" (p. 9, l.318 – 323) (Porcine tissue was chosen, as it is a common animal model in cardiovascular medicine and it is difficult to obtain non-pathologic human samples for tensile testing.)

2. *Line 46-47: An appropriate material was chosen and specific material thicknesses were defined.*

   Comments: The material's name needs to be mentioned specifically, as well as the defined thickness. It looks like the material is printed by the Polyjet printer, and from the imaging results, it is Agilus 30, but in the paper, the name of this flexible 3D printing material is not mentioned, which needs to highlighted instead.

   Authors*:* The name of the printing material is Keyence AR-G1L, which is printed using the Polyjet technology. According to the journals submission guidelines, we added it to the table of materials (s. guidelines in the manuscript template: "*Avoid the use of commercial language, including ™/®/© symbols or company brand names before/after an instrument or reagent. Cite these in the Table of Materials instead.*").

3. *Line 52: Contrast medium was used to enhance visibility in x-ray-based imaging. Comments: What is the name of the contrast medium? By the way, this problem seems to happen throughout the paper, when the author illustrate the experimental methodology, material's names are missing.*

   Authors*:* We thank the author for his important note. We fully agree that mentioning explicit names and products should be part of a journal's method section. Similar to the printing material, the exact name of the contrast agent had to be excluded from the manuscript and is therefore added to the table of materials.

4. *Line 56: Mechanical properties were comparable to physiological biomechanics.*

   Comments: What mechanical properties? For example, Young's modulus, or shear stress, etc? Also in the following main sections, the mechanical comparison results need to be added to prove it is actually comparable.

   Authors*:* The printing material was compared to porcine aortic tissue samples using tensile tests. Different material thicknesses were tested to achieve a maximum tensile strength and elongation comparable to the results of porcine aortae. These results are representative for the shown aortic aneurysm model but will differ for other cardiovascular structures (i.e. ventricular or atrial wall). We added the tensile test results to the representative results section: (p. 9, l.318 – 323).

5. *Line 57-58: Printed models were permeable for x-ray as well as ultrasound and visualization was similar to human tissue.*

   Comments: For tissue-mimicking materials for ultrasound and X-ray, it is recommended that the following papers can be cited: "…"

   Authors*:* The authors thank the reviewer for his valuable input and the suggested publications. The development of tissue-mimicking materials is invaluable for the improvement of in vitro

models. Therefore, we added a section regarding tissue mimicking material development to the discussion section, citing the above mentioned publications: "*Creating tissue mimicking materials for 3D-printing is still a focus of scientific research and the development of novel materials will results in even more realistic results*." (p: 11, l: 391-393)

6. *Line 61: patient-specific phantoms were easy to catheterize and provided a realistic training modality.*

   Comments: What is the ground-truth model here to support the accuracy of the phantoms. To prove the phantoms are patient-specific and realistic, the comparison results between the ground-truth, segmentation and printed phantoms need to be added as well, for example, error percentage.

   Authors*:* Materialise Mimics offers the option to overlap the final digital model with the underlying patient scan. This is used on every model, created with the described process. Like this, the accurate representation of anatomical details in the final model can be ensured.

   Furthermore, all training models were tested and catheterized by experienced cardiologist, as well as medical students. Preliminary feedback was collected in a questionnaire which showed a high degree of realism. The data collected during the tests will be published in a doctoral thesis which is why we unfortunately had to exclude it from the manuscript.

7. *Line 86-87: The aim of this study was to develop a method for individualized therapy planning in cardiovascular medicine by generating patient-specific or disease-specific phantoms.*

   Comments: From the shown results, it is more like a phantom developing procedure instead of a new method.

   Authors*:* The authors want to excuse for the misleading wording. We acknowledge that a more precise description is required in describing the aim of this methodological publication. Therefore, we adjusted the sentence as follows: "*The aim of this study was to establish a method for generating patient-specific or disease-specific phantoms, usable in individualized therapy planning in cardiovascular medicine."* (p.3 l: 84- 85)

8. *Line 96-98: The pixel size and slice thickness (ST) of the dataset should be adapted to the size of the structures intended to be represented in the 3D model.*

   Comments: What is the voxel size and slice thickness of the CT dataset?

   Authors*:* The datasets utilized in this study are isotropic with a slice thickness of 1mm resulting in a voxel size of $1mm^3$. The authors added this information to the "representative results" paragraph: "*The presented results were created using isotropic CT-datasets with a ST of 1mm and a voxel size of $1mm^3$."* (p. 9, l: 319-321)

9. *Line 125-127: If the slice thickness is too thin, the required computing power for modelling will increase severely, which also slows the process significantly.*

   Comments: Is there any advantage of thin slice thickness? Will it improve the resolution? Because from the results, the resolution of the CT dataset is very low, which will affect the segmentation.

   Authors*:* The authors thank the reviewer for highlighting this important point. The required slice thickness depends heavily on the anatomical structures that should be represented in the final model. The authors agree that a low-resolution dataset will result in poor segmentation

results, especially for small diameter vessels. The manuscript was adjusted to also include this fact: *"On the other hand, if the slice thickness is too high, small details in the patients' anatomy might get lost." (p. 4 l. 126-128)*

10. *Line 140: A range of Hounsfield unit values is then defined…`*

    Comments: What is the range of Hounsfield unit.

    Authors*:* The Hounsfield unit range slightly depends on the underlying CT-dataset. For example, in contrasted CT-scans we use a HU range of roughly 200-800 HU to segment the vessels. This information has been added to the manuscript to give the reader a general idea of where to start the segmentation process: "*…a range of Hounsfield unit values is then defined (approx. 200-800 HU), resulting in a combined mask of the contrast-enhanced blood volume and bone structures (e.g. sternum, parts of the ribcage and spine)."* (p. 5 l: 141-143)

11. *Line 181-182: This step allows the selection of a fixed wall thickness for the entire model.*

    Comments: What is the wall thickness of the segmented model, will the chosen of thickness affect the pumping experiment, for example, the bulgy out problem?

    Authors*:* The wall thickness of the model presented in the representative results is 2.5 mm. Since the wall thickness will have a direct impact on the flexibility and response to mechanical stress, a change in wall thickness of printing material will lead to differing results from the ones presented. Therefore, the desired mechanical properties should be recreated in the region of interest of the model. In the described setting, both submerged and exposed models showed no bulging out during pulsatile flow experiments. We added this information to the Note explaining the impact of the chosen wall thickness on the model's mechanical behavior. " Since the wall thickness is constant across the entire model, the desired mechanical properties should be recreated in the region of interest of the model." (p. 6; l:190-191) and: "…mechanical properties of the phantom can be adjusted by variations of the wall thicknesses, as it was done in this study. This is why it is not recommendable to only duplicate the thickness of the tissue of interest from the underlying tomographic data." (p. 11; l:395-397)

12. *Line 196-198: NOTE: To confirm the accuracy of the designed 3D-model, some software enable the overlay of the final STL's contour and the underlying radiological dataset. This allows a visual comparison of the 3D-model to the native anatomy.*

    Comments: What are the software to confirm the 3D model accuracy? What are the error percentage of the model created in this paper after comparison? Ambiguous illustration needs to be replaced by accurate data and software names.

    Authors*:* Materialise Mimics offers the option to overlap the final digital model with the underlying CT-scan. This is used on every model we create in the Mimics Innovation Suite to ensure the accurate representation of anatomical details in the final model. From a 3D-printing perspective, the used Keyence Agilista printer offers a spatial resolution of 30µm for flexible models. We added the information on the 3D-printer, including spatial resolution to the table of materials. While this only offers a qualitative rather than a quantitative analysis, the authors believe that the resulting models reach an anatomical accuracy that will be ideal for surgical planning and interventional training. We adjusted the note to better reflect the need for a high-resolution printer in addition to the specific software: "*Furthermore, a printer with a suitable spatial resolution of < 40 µm has to be selected to allow for an accurate print of the digital model."* (p. 6; l: 201-203)

13. *Line 209-211: followed by immersion in water or a respective solvent (depending on printing material) and a final drying step in an incubator.*

    Comments: What are the solvents used in this paper?

    Authors*:* We excuse the imprecise wording in this sentence! The support material Keyence AR-S1 is water soluble. Since the availability of printing materials and printing techniques differs for every research group, the authors tried to keep this aspect rather broad, to increase the accessibility of this method to different printing techniques.

14. *Line 215-216: Then, agar has to be heat-dissolved and poured into the box to create a bed of at least 2 cm height to place the model on.*

    Comments : What is the temperature and heating tool to melt agar? How long the agar will become solid again once cooled down to room temperature? The specific temperature and heating tool need to be mentioned. And compared to water environment, what are the advantage and disadvantage of using agar environment? As a lot of people also use water as the ultrasound imaging environment.

    Authors*:* The agar-water mixture is boiled briefly, preferably on a stovetop or in a microwave. After pouring it into the box, it will take roughly an hour for the agar to fully set and cool to room temperature. We adjusted the paragraphs regarding the instructions for preparing the agar accordingly: "*Then, an agar solution (1% in water) has to be prepared and brought to a boil. After boiling and stirring the mixture, let it cool for 5 minutes and pour into the box to create a bed of at least 2 cm height to place the model on.*"(p. 7 l: 220-223)

    "*Finally, it will take about an hour at room temperature for the agar to fully cool and set*." (p.: 7; l: 238-240)

    Agar offers a few benefits compared to submerging the model in water environment. First of all it reduces movements artifacts in CT or MRI scans, caused by the pulsatility of the flow loop. Secondly, it gives a better haptic feedback during sonographic imaging and a better force feedback during catheterization. We adjusted a note, giving the reader a better understanding of why we chose Agar as an embedding substance. "The embedding of the model offers two benefits. First, it reduces movement artifacts during clinical imaging of the model. Secondly, agar offers a better haptic feedback during sonographic imaging, and a more realistic force feedback during catheterization compared to a submersion in water." (p. 7 l.224 – 227)

15. *Line 281: hardware and software (e.g. C-arm and imaging software)*

    Comments: What is the imaging software used in this paper?

    Authors*:* We use the standard Siemens imaging software *syngo via,* available at the radiological department. As with the materials mentioned above, unfortunately we can't add this to the manuscript, but detailed information can now be found in the table of materials.

16. *Line 285-286: wires and catheters are visible on the screen just like during a real patient intervention.*

    Comments: What are the names of the wires? For example, sheath, etc. What are the names of the catheters? For example, ablation catheters. The author keeps using ambiguous words to describe the experiments and materials, which should be avoided in a scientific paper.

Authors*:* Depending on the model and type of intervention, standard clinical guidewires and catheters were used. For implanting (T)EVAR prostheses, we use a *Lunderquist* guidewire. For catheterization trainings in congenital heart defect models we use diagnostic catheters in sizes 4F and 5F by Cordis. Furthermore, we use guidewires by Angiokard GmbH or Terumo Europe NV with a diameter of 0.035". All material information was added to the table of materials to give the reader a better understanding of the required catheterization materials.

17. *Line 294-295: Modification of the 3D printed models in the 3D-modeling software allows the adaptation of the model structure to any training level and goals.*

    Comments: What 3D modelling software is used in this paper as modification tool? For example, Meshmixer, Blender, etc?

    Authors*:* The 3D-modeling software of the Materialise Mimics innovation suite, 3-matic is used to modify the anatomical models. Additional components are designed with a standard CAD software "Solidworks". The name and version number of the 3D-modeling software can be found in the revised table of materials.

18. *Line 325-326: The printed material can easily be distinguished from the surrounding agar and possible metallic implants.*

    Comments: Use the name of the actual printed material to replace, for example, Agilus30.

    Authors*:* We understand the reviewer's concerns, however we are not allowed to include brand names in the manuscript. Therefore, these information can be found in the table of materials now. The printing material used in this study is Keyence AR-G1L.

<u>Reviewer 2 Comments</u>

Authors*:* The authors thank the reviewer for his careful and insightful review of our manuscript. We addressed all concerns in the following paragraphs. Where applicable we added the adjusted manuscript lines or a referral to the table of materials.

1. *"The appearance of the 3D models under X-ray imaging as well as the haptic feedback from the manipulation of the instruments inside the model were assessed as extremely realistic. Repetitive training on 3D models leads to well-versed orientation in 3D, improved perception of haptic feedback and - most important for the patient - minimization of radiation exposure." Although I agree with this sentence, do you have some data to back up this claim? Satisfaction questionnaires? Literature review? It is important to quantify how realistic is the model, in an objective way.*

   Authors: The authors thank the reviewer for this important comment. The training setups were initially tested by experienced cardiologists and subsequently by medical students and assistant doctors. The overall experience was rated excellent in currently unpublished survey data, which will be published soon as part of a doctoral thesis. While this is a subjective assessment of the model's quality, we believe the models prove to be realistic and ideal for training medical personnel in the use of interventional techniques.

2. *The protocol using Mimics is well detailed. However, for broader application of this protocol, please consider explaining the steps using 3D Slicer, as it is a free software and therefore more widespread.*

   Authors*:* The authors thank the reviewer for highlighting this point. While we agree, that an additional protocol to explain every step in 3DSlicer might be beneficial, it would increase the ambiguity of the manuscript and make it harder to follow the instructions. Therefore, we refrain from adding additional command names and protocol steps that might lead to confusion. 3DSlicer is an excellent freeware solution for segmenting radiological datasets. But, same with most freeware programs, it requires a high amount of initial work to get the program adjusted to the individual needs. Furthermore, it is not possible to modify the 3D-models in 3DSlicer but rather they have to be transferred to a different freeware modeling software such as Blender. In contrast to the Materialise Mimics Innovation Suite, which unifies all these steps in a build in software solution, the use of 3DSlicer and an additional modeling software will be filled with very individual issues, that can't be fully covered in the manuscript format of the JoVE. We added this very valuable aspect to the discussion of the manuscript and hope it offers a satisfactory answer to your comment above: "*The freeware options offer excellent results when creating 3D-models from radiological datasets, although a high amount of initial work is required to adjust the software to individual needs. Furthermore, a subsequent manipulation of the digital model requires an additional software, which is why a comprehensive software suite covering all these aspects is highly recommended for a quick and smooth workflow.*" (p: 12, l: 420 – 424)

3. *In the 3D printing section, you use an Agar set. Can you elaborate why? Advantages compared to other agents?*

   Authors*:* The reviewer mentioned a very important point. Agar offers a few benefits compared to submerging the model in water environment. First of all, it reduces movements artifacts in CT or MRI scans, caused by the pulsatility of the flow loop. Secondly, it gives a better haptic feedback during sonographic imaging and a better force feedback during catheterization. We tried a few different materials for covering the model, (i.e. water and jellylike paraffin wax). Agar offered the overall best properties for use with our 3D-printed models, since water didn't give the right haptic feedback and showed poor results in reducing motion artifacts in CT and MRI imaging. Due to the composition of the printing material, the jellylike paraffin wax was not suitable. Since it slowly dissolved the printed anatomies, making it an unreliable agent, even for short term use. We adjusted the respective NOTE to offer the reader a better understanding of why agar was chosen as an embedding material: "*NOTE: The embedding of the model offers two benefits. First, it reduces movement artifacts during clinical imaging of the model. Secondly, agar offers a better haptic feedback during sonographic imaging, and a more realistic force feedback during catheterization compared to a submersion in water*." (l: p. 7 l. 224-227)

4. *Some sentences require syntax editing, for instance: "Segmentation of medical images is a Hounsfield- based of pixels based on their Hounsfield units to form 3-dimensional models."*

   Authors*:* We apologize for the oversight of this obvious mistake and we corrected the sentence accordingly. Furthermore, the whole manuscript was revised in terms of language and syntax. *"Segmenation of medical images is based on their Hounsfield units to form 3-dimensional models*." *(p. 4 l: 131-132)*

5. *Can you elaborate on the applications of this technology? From echo, CT/MR and invasive training, which are the ones with greater potential and applicability in the future? Which one is more cost-effective?*

   Authors*:* We thank the reviewer for this important comment! Since this is a key question and aspect of an innovative method, we added this outlook to the discussion: "*The cost-effectiveness of 3D-printed models can be very controversial and highly dependent on the material properties. In interventional training a high durability of each model, even after balloon dilation is required to reduce the overall costs. When looking at patient-specific therapy planning, one must mainly look at the beneficial effect of a printed model. A 3D-printed model won't be cost-effective for a "standard" surgical patient, but might offer tremendous insight in patients with complex anatomies. Therefore, the costs of training models have to be always weighed against their proposed benefit."* (p: 12, l: 404-410)

   "*The presented method can be beneficial for a wide range of clinical and basic research fields. The use of realistic models is excellent for teaching young doctors and students the basics of sonography as well as interventional techniques. Especially with interventions, such a model will make the technology more accessible and increase the overall knowledge base of doctors, longterm. CT and MRI imaging, especially when looking at hemodynamic flow patterns in the aortic vessels, can be a big addition both in basic science, as well as determining the outcome of surgical and transcatheter interventions."* (p: 12, l: 438-445)

Figure 1 Revised table of materials

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
3-matic	Materialise AB		Software Version 15.0
Affiniti 50	Philips Medical Systems GmbH		Ultrasonic Imaging System
Agilista W3200	Keyence Co.		Polyjet 3D-Printer with a spatial resolution of 30µm
AR-G1L	Keyence Co.		flexible 3D-Printing material
Artis Zee	Siemens Healthcare GmbH		Angiographic X-ray Scanner
cvi42	CCI Inc.		Software Version 5.12 - 4D Flow Analysis Software
Diagnostic Catheter, Multipurpose MPA 2	Cordis, A Cardinal Health company		Catheter for pediatric training models, Sizes 4F for infants and 5F for children, young adults
Excor Ventricular Assist Device	Berlin Heart GmbH		80 -100ml stroke volume
Imeron 400 Contrast Agent	Bracco Imaging		CT - Contrast Agent
IntroGuide F	Angiokard Medizintechnik GmbH		Guidewire with J-tip; diameter: 0.035" length: 220cm
Lunderquist Guidewire	Cook Medical Inc.		(T)EVAR interventional guidewire
MAGNETOM Aera	Siemens Healthcare GmbH		MRI Scanner
Magnevist Contrast Agent	Bayer Vital GmbH		MRI - Contrast Agent
Mimics	Materialise AB		Software Version 23.0
PVC tubing			
Radifocus Guide Wire M	Terumo Europe NV		Straight guidewire; diameter: 0.035" length: 260cm
Really useful box 9L	Really useful products Ltd.		
Rotigarose - Standard Agar	Carl Roth GmbH	3810.4	
Solidworks	Dassault Systemes SE		Software Version 2019-2020; CAD Design Software
SOMATOM Force	Siemens Healthcare GmbH		Computed Tomography Scanner
syngo via	Siemens Healthcare GmbH		Radiological Imaging Software