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Porcine liver vascular bed in Biodur E20 corrosion casts

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Background: *Pigs are frequently used as animal models in experimental medicine. To identify processes of vascular development or regression, vascular elements must be recognised and quantified in a three-dimensional (3D) arrangement. Vascular corrosion casts enable the creation of 3D replicas of vascular trees. The aim of our study was to identify suitable casting media and optimise the protocol for porcine liver vascular corrosion casting.*

Materials and methods: *Mercox II® (Ladd Research, Williston, Vermont, USA) and Biodur E20® Plus (Biodur Products, Heidelberg, Germany) were tested in 4 porcine livers. The resins (volume approximately 700 mL) were injected via the portal vein. Corrosion casts were examined by macro-computed tomography, micro-computed tomography and scanning electron microscopy.*

Results: *For hepatectomies, the operating protocol was optimised to avoid gas or blood clot embolisation. We present a protocol for porcine liver vascular bed casting based on corrosion specimens prepared using Biodur E20® epoxy resin.*

Conclusions: *Only Biodur E20® Plus appeared to be suitable for high-volume vascular corrosion casting due to its optimal permeability, sufficient processing time and minimum fragility. Biodur E20® Plus is slightly elastic, radio-opaque and alcohol-resistant. These properties make this acrylic resin suitable for not only vascular research but also teaching purposes.* (Folia Morphol 2016; 75, 2: 154–161)

Key words: porcine liver, vascular corrosion cast, Biodur E20, micro-computed tomography, scanning electron microscopy

INTRODUCTION

Because the anatomical, genetic and physiological characteristics of pigs are similar to those of humans [33], pigs are frequently used as animal models in experimental medicine. The liver parenchyma has the unique ability to regenerate [14]. Its functional capacity is dependent on establishing new microcirculation [9]. Detailed anatomical

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Table 1. Mammalian liver vascular corrosion casts — review, animals arranged in alphabetical order

Animal	Resin	Injected by	Pressure monitored	Micro-vasculature filled	Reference
Cow	Dental acron*, Technovit (*Heraeus Kulzer, Hanau, Germany*)	PV, HA, hepatic veins, hepatic ducts	*	No	[30]
Dog	Polirepar S (*Polident, Dental Products Industry, Slovenia*)	PV, HA, CCV, bile duct	No (manual injection)	No	[32]
	Epoxy resin*, polymerising agent AralditeND*	PV	*	No	[26]
Human	Batson's #17 (*Polysciences, Warrington, USA*)	PV, HA	Yes	Yes	[5–7]
	Polirepar S (*Polident, Volčja draga, Slovenia*)	PV, HA, hepatic ducts, vena cava inferior	No (manual injection)	No	[4]
	Mercox (*Oken-Shoji, Tokyo, Japan*)	PV, HA	*	Yes	[28]
Mouse embryo	Mercox (*Dainnipon Ink Co.*)	Umbilical vessel*	Yes	Yes	[16]
Monkey	Mercox (*Oken-Shoji, Tokyo, Japan*)	Thoracic aorta	*	Yes	[28]
Pig	Technovit 7143 (*Heraeus, Germany*)	PV	*	No	[23]
Rabbit	Mercox (Oken-Shoji, Tokyo, Japan)	Thoracic aorta	*	Yes	[28]
Rat	Mercox CL2 R (*Okenshoji, Tokyo, Japan*)	Ascending aorta	Yes	Yes	[10]
	Mercox (*Oken-Shoji, Tokyo, Japan*)	Thoracic aorta	*	Yes	[28]
Ship	Interacryl cold (*Interdent, Gornji Grad, Slovenia*)	PV, HA, hepatic veins	*	No	[12]

*Not specified; CCV — caudal caval vein; HA — hepatic artery; PV — portal vein

data for the liver microvasculature are required to identify processes of vascular development or regression [17], for quantitative analysis [18, 19, 31] or for blood-flow modelling [5, 29]. Vascular corrosion casts enable the creation of three-dimensional (3D) replicas of vascular trees.

The liver has a unique blood flow pattern. There are two inputs: the portal vein (PV) and the hepatic artery (HA). The PV transmits nutrient-rich, less oxygenated, low-pressure blood from unpaired organs in the abdomen. The HA inflow is the second, smaller (approximately 30%) input for the liver and conveys oxygenated blood. The PV and HA enter the porta hepatis, they divide into the right and left branches and subsequently the segmental and subsegmental branches. The arterial branches invariably follow those of the PV [3]. Their terminal branches end in the hepatic sinusoids and the peribiliary and periportal plexuses, which open into the portal vein or hepatic sinusoids [28]. The interlobular (collecting) branches of the portal vein continue into the circumlobular (distributing) branches and hepatic sinusoids [8, 15]. Blood from the sinusoids enters the hepatic veins via the central veins. The hepatic veins drain into the caudal caval vein (CCV). This complicated liver vasculature makes liver corrosion casting challenging, particularly in larger animals. The porcine liver microvasculature has not yet been studied from this perspective (Table 1). The aim of our study was to identify suitable casting media and optimise the protocol for porcine liver vascular corrosion casting.

MATERIALS AND METHODS

For the corrosion casting of livers, 4 *Sus scrofa* f. domestica piglets (age 42–70 days, weight 12–25 kg) were operated on under general anaesthesia.

Casting protocol

Casting protocol including: hepatectomy, casting media dilution and resin injection, polymerisation and maceration.

Hepatectomy. The piglets were premedicated intramuscularly with atropine 1.0 mg, ketamine 200 mg (approximately 5–10 mg/kg) and azaperon 160 mg (2–8 mg/kg). Anaesthesia was administered continuously through a peripheral or central venous catheter at the following total average doses: propofol (1% mixture 5–10 mg/kg/h) and fentanyl (1–2 μg/kg/h). Muscle relaxation was ensured by bolus administration of pancuronium 0.1–0.2 mg/kg at the beginning of the surgery. The piglets were intubated and mechanically ventilated during the surgical procedure and received infusions and volume substitutions when necessary (Plasmalyte Baxter and Gelofusine B-Braun, respectively). Electrocardiogram, oxygen saturation and central venous pressure were monitored during surgery. The surgical procedure was performed under aseptic and antiseptic conditions. To maintain free macro- and micro-circulation to fill the entire vascular bed, heparin (30,000 UI i.v.) was administered. Before hepatectomy, the vascular tree was flushed with 5 L of heparinised solution (dilution: 10,000 UI of heparin per 1 L of Hartmann's solution) via the caudal caval vein. To prevent gas emboli, the HA, PV, hepatic ducts and CCV were cut under water niveau. After washing, the liver vascular system was again clamped. The piglets were sacrificed at the end of the operation under deep general anaesthesia with a concentrated solution of potassium chloride administered into a central vein.

The experimental surgical and anaesthesiological procedures and the use of piglets in this study were certified by the Commission for Work with Experimental Animals at the Pilsen Medical Faculty of Charles University, Prague and were under the control of the Ministry of Agriculture of the Czech Republic. All procedures were prepared and performed under the law of the Czech Republic, which is compatible with the legislation of the European Union.

Casting media. For porcine livers of animals weighing less than 30 kg, 500–700 mL of resin appeared to be satisfactory. The injection volume of the resin is dependent on the size of the liver. Leakage of the resin from the CCV indicated that the liver was adequately filled. Because polymerisation begins as soon as the resin is mixed with the catalyst, all preparations involving the application of the resin must be completed prior to mixing.

Figure 1. Yellow-dyed Biodur E20® Plus manual injection into the portal vein under water niveau.

Mercox II® (Ladd Research, Williston, Vermont, USA): 400 mL of Mercox II was mixed with 9 g of catalyst (40% benzoyl peroxide).

Biodur E20® Plus (Biodur Products, Heidelberg, Germany): 400 g of Biodur E20 Plus was mixed with 180 g of catalyst E2(0).

Direct contact with the highly toxic chemicals was avoided by working in a ventilated room and wearing protective gloves, glasses and respiratory filters.

Resin injection, polymerisation and maceration. To prevent artefact formation during hardening, the liver was positioned upwards with the diaphragmatic surface placed in a container filled with lukewarm tap water. To avoid air embolisation, all of the procedures were performed under the surface of the water. After releasing all vessel ligations, resin was injected under manual pressure (flow rate 150 mL/min) into the portal vein via a Nelaton catheter (Fig. 1). Once resin began leaking from the caudal caval vein, the PV, HA and inferior vena cava were clamped. Then, the liver was stored in the water and allowed to polymerise at room temperature for the next 24 h. The liver was then macerated for 3 days at room temperature in a 15% potassium hydroxide solution, and the cast was rinsed in tap water. The whole-liver casts were preserved in 90% alcohol.

Computed tomography (CT) scanning and data processing

1. The casts were first scanned in 90% alcohol using a multi-slice human CT (Somatom Sensation 64, Siemens, Forchheim, Germany) with a slice thickness of 0.6 mm and voxel size of 0.4 × 0.4 × × 0.6 mm.

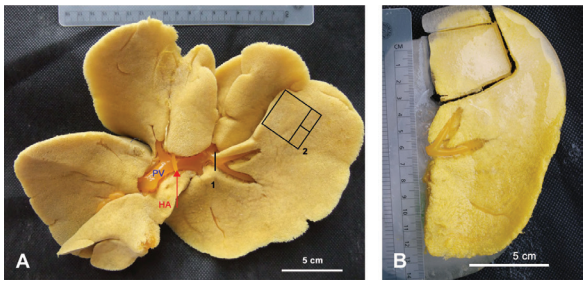

Figure 2. **A.** Porcine liver vascular corrosion cast, visceral surface, portal filling with yellow-dyed Biodur E20® Plus (Biodur products, Heidelberg, Germany); PV — portal vein; HA — hepatic artery proper; **B.** Left lateral lobe — segmentation in an ice prism.

2. To perform high-resolution scanning, the casts were cut into small prisms (Fig. 2). The following sampling pattern was used: Each lobe was separated, rinsed of alcohol and frozen in distilled water, and prisms approximately 2 × 1 × 1 cm in size (Table 2) were randomly cut with a belt saw. Randomly chosen samples underwent micro-CT examination.
3. The microarchitecture of the liver vessels at the level of the sinusoids was scanned in air using a micro-CT (Xradia XCT 400, Pleasanton, CA, USA). The pixel sizes used for imaging were 17 μm, 9.5 μm and 4.5 μm. The architecture of the liver vessels at the level of the sinusoids was visualised using a volume rendering technique and thin-slab maximum intensity projection.

Scanning electron microscopy (SEM)

The remaining cut lobes were placed into distilled water. They were frozen and cut with a belt saw into prisms approximately 1 × 1 × 0.5 cm in size. Specimens were cleaned in 5% formic acid for 20 min, rinsed in distilled water and re-frozen. After freeze--drying, the specimens were mounted on copper foils and fixed using wire stubs with conductive silver paste according to a method described by Lametschwandtner et al. [21]. Then, the specimens were sputtered with gold for 60 s and examined using a Stereoscan 250 SEM (Cambridge, UK) at an accelerating voltage of 10 kV. Preliminary imaging was performed with an SEM SU-70 (Hitachi, Japan) using an accelerating voltage of 1 kV because this protocol does not require any pretreatment of specimens.

RESULTS

The differences between the two resins tested are summarised in Table 3. Mercox II® (Ladd Research, Williston, Vermont, USA) did not appear to be suitable for casting volumes greater than 400 mL. The main disadvantage was the highly variable, short working time, which is dependent on the quantity of resin and storage. For greater quantities, the working time rapidly decreases, and mixing produces a considerably exothermic reaction.

However, in all three liver casts, Biodur E20® Plus (Biodur Products, Heidelberg, Germany) passed through the portal venous bed and also filled the sinusoids and hepatic venous system (Figs. 3, 4). In the smallest liver, the HA proper was also retrogradely filled (Fig. 2A). Due to its chemical and rheological properties, Biodur E20® Plus was thus suitable for large-volume corrosion casting and provided good replication quality (Fig. 4C). In macro-CT, the software packages permitted the separation of the caval from the portal systems (Fig. 5). The radio-opacity of Biodur E20® Plus also permits its use for high-resolution CT imaging (Fig. 3).

In all casts and samples, we observed globular structures (Fig. 3) in course of sinusoids that appeared under SEM to be resin extravasations (Fig. 4D).

DISCUSSION

To obtain the highest authenticity 3D vascular bed in corrosion casts, careful preparation, specific anaesthesia and irrigation of the arterio-venous tree

Table 2. Review of Biodur E20® Plus porcine liver samples examined

Liver	Weight of pig	Lobe cut	Micro-CT 2 × 1 × 1 cm	SEM 1 × 1 × 0.5 cm
No. 1	12 kg	Left lateral lobe	2	2
		Right medial lobe	2	2
No. 2	27 kg	Right lateral lobe	3	4
No. 3	25 kg	Right lateral lobe	3	4

CT — computed tomography; SEM — scanning electron microscopy

Table 3. Resins tested

Name of resin, producer	Number of whole liver casts	Chemical nature of resin	Dilution used		Working time	Other properties
			Resin	Catalyst		
Mercox II® *Ladd Research, Williston, Vermont, USA*	1	Acrylic	400 mL of Mercox II®	9 g	5 min (max.)	Advantages: sufficient radio--opacity for micro-computed tomography imaging (Fig. 6) Disadvantages: a short, variable working time; a maximum appropriate mixing volume of only 20 mL; very fragile, difficult-to-cut cured product. Casts must not be preserved in alcohol!
Biodur E20® Plus *Biodur Products, Heidelberg, Germany*	3	Epoxide	400 g of E20	180 g of E2(0)	40 min (min.)	Advantages: a long working time; sufficient radio-opacity (both the radio-opacity and the processing can be increased by commercially available Biodur additives); low cost; alcohol resistant; slightly flexible

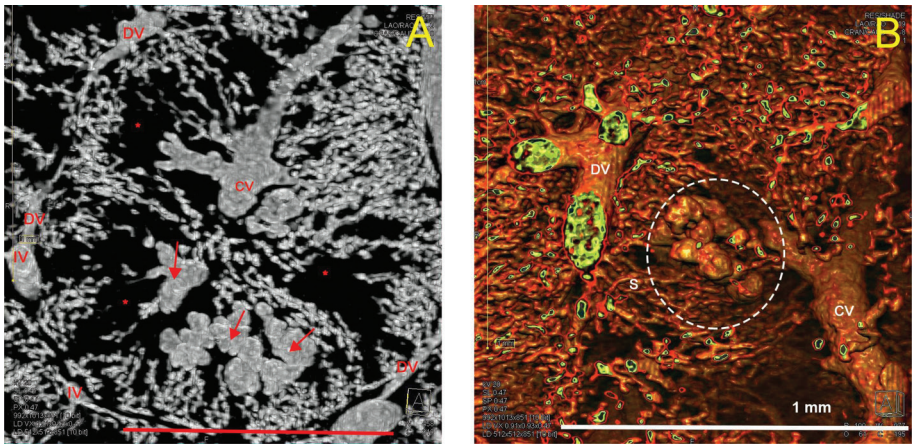

Figure 3. A. Porcine liver vascular corrosion cast, portal filling, Biodur E20® Plus, maximum intensity projection reconstruction; CV — central vein; DV — distributing portal vein; IV — interlobular (conducting) portal vein; S — sinusoid; arrow — resin extravasation; asterisk (*) — incomplete sinusoidal filling; micro-computed tomography, bar 1 mm; **B.** Porcine liver vascular corrosion cast, portal filling, Biodur E20® Plus, volume rendering technique reconstruction; CV — central vein, DV — distributing (circumlobular) portal vein; S — sinusoid; circle — resin extravasation; micro-computed tomography.

are necessary. The choice of suitable casting material is determined by a variety of factors, such as the purpose of the final casts, cast size and the modality of subsequent examination. The casting medium should have adequate viscosity (to pass through but not penetrate), and sufficient working time is limiting, especially in large casts. Additionally, the casting material must be capable of even polymerisation with minimal shrinkage and physicochemically resistant to further corrosion, dissection and preservation procedures [11, 20].

The most commonly used resins (Table 1) are methyl methacrylates such as Mercox [2] or modified Batson's 17 [5, 6]. Methyl methacrylates are generally capillary-passable and alcohol non-resistant. Their limitations are a relatively short working time and high fragility [11]. Biodur E20 is a translucent, firm epoxy resin of medium viscosity and slightly flexible consistency. It was used by Masset et al. [24] to reveal the angioarchitecture of equine periodontium. With the exception of shrinkage, which was not assessed in our study, Biodur E20® Plus appeared to be suitable for large

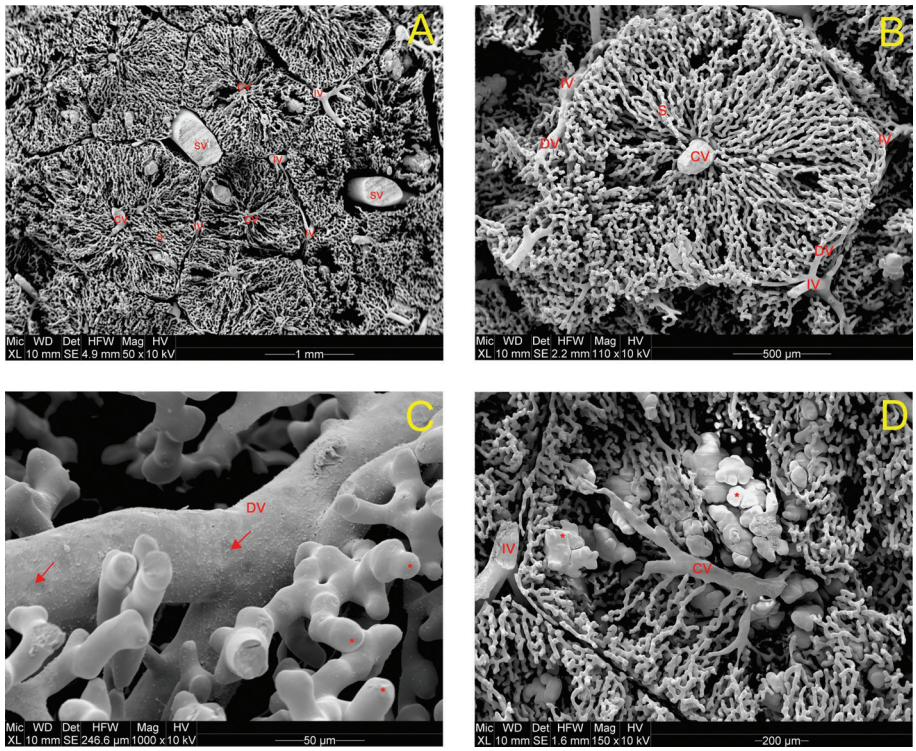

Figure 4. Porcine liver vascular corrosion cast, portal filling, Biodur E20® Plus; scanning electron microscopy; **A.** CV — central vein; DV — distributing portal vein; IV — interlobular (conducting) portal vein; S — sinusoids; **B.** Hepatic lobule; CV — central vein; S — sinusoids; DV — distributing portal vein; IV — interlobular (conducting) portal vein; SEM; **C.** DV — distributing (circumlobular) portal vein; asterisk (*) — blindly ending sinusoids; arrow — imprints of endothelial cell nuclei; **D.** Hepatic lobule; CV — central vein; IV — interlobular (conducting) vein; asterisk (*) — resin extravasation.

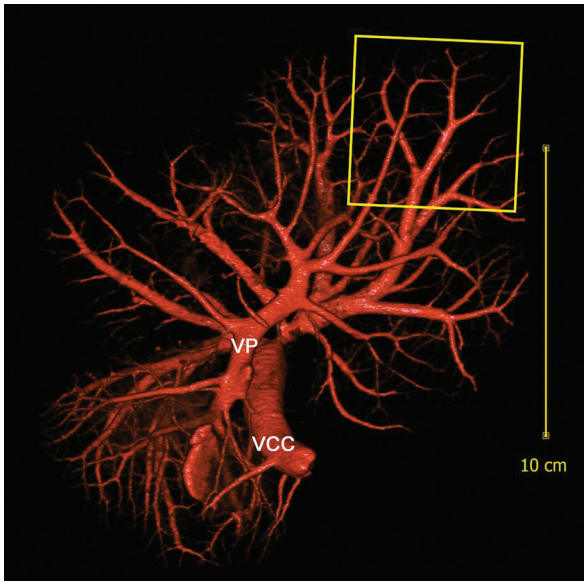

Figure 5. Porcine liver vascular corrosion cast, portal filling, Biodur E20® Plus, volume rendering technique; VP — portal vein; VCC — caudal caval vein, rectangle — cut part of the left lateral lobe; multi-slice human computed tomography.

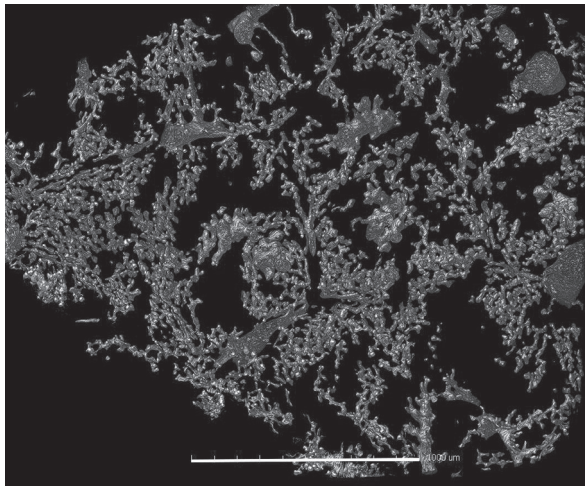

Figure 6. Porcine liver vascular corrosion cast, portal filling, Mercox II®; micro-computed tomography, bar 1 mm.

vascular corrosion casts. Although the resin was injected only via the portal vein, both micro-CT and SEM scans demonstrated that Biodur E20® Plus passed through the

sinusoids and connected the portal and hepatic veins (Figs. 3, 4). However, the arterial beds with peribiliary plexuses [28] in the interlobular spaces were missing, and parts of the lobules and sinusoids were not completely filled (Figs. 3A, 4A, B). These absences might have been due to a contraction of inlet sphincters, by cells protruding into the sinusoidal lumen [25] or by the absence of pressure from the missing arterial inflow.

To our knowledge, a liver corrosion cast of a specimen weighing over 20 kg has not before been created (Table 1). The only exception is a human liver discarded for transplantation that was casted using Batson's 17 [6, 7]. Batson's 17 appeared to be suitable for CT scanning, but the quality of the microvessel bed replica has only been demonstrated on a small-volume sample.

In all of our samples (Table 2), the micro-CT scans revealed globular structures in the sinusoids, which could have been either tortuous sinusoids or artefacts (Fig. 3). SEM, which permits the identification of delicate structures such as vessel sphincters, valves, and nuclear imprints [1, 27], suggested that these grape-like structures were resin extravasations, i.e., artefacts (Fig. 4D). In our study, we did not monitor the pressure during injection because pressure does not correspond to the peripheral intraluminal pressure. Because of the size of the livers and the rheological properties of Biodur E20® Plus at 20°C (kinematic viscosity 150–170 cST, density 1.1 g/cm^3), the extravasates were likely the result of local higher sinusoidal permeability because the liver sinusoids are lined by discontinuous endothelial cells with fenestrations and the basal lamina is missing [22]. The globular shape of the extravasates might be due to the hydrophobic nature of the epoxy resin as the space of Disse was filled with a water solution during the hepatectomy.

Except for a few organs [13], to make a microvascular corrosion cast, the entire, fresh organ must be available. This condition highlights the importance of our results, as pigs are frequently used as animal models in experimental medicine. In addition, the alcohol resistance of Biodur E20® Plus with the possibility of soft tissue preservation increases the potential of the application of casts made with this resin for not only research but also teaching purposes [11].

CONCLUSIONS

We present a protocol for porcine liver vascular bed casting based on corrosion specimens prepared using Biodur E20® Plus epoxy resin (Biodur Products, Heidelberg, Germany). Biodur E20® Plus permeates throughout the entire microvascular bed and offers sufficient radio-opacity. The degree of elasticity of Biodur E20® Plus permits safe cast handling as well as dissection, and the alcohol resistance of this resin is useful for the preservation of specimens both before and after corrosion. Macro- and micro-CT scans enabled 3D reconstruction of the entire vascular bed. The micro-CT images enabled stereological assessment. In combination with SEM, corrosion casting increases the possibility of morphometric analysis of a vascular network in health and disease. Our results can also be used to correlate 3D models with 2D liver histopathology or to provide data to optimise liver resections or perfusion models. Because pigs are frequently used as a surrogate animal model, our results can be used for not only morphological vascular research but also anatomy education.

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