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Use of water-soluble dye in antiseptic fixed cadaveric vascular mold silicone injection

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Research Article

Keywords: cadaveric, specimen, dissection, silicone, injection, dye

Posted Date: June 29th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9884284/v1

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Anatomical Science International on September 11th, 2026. See the published version at https://doi.org/10.1007/s12565-026-00969-z.

Abstract

Purpose

The simple, rapid, and inexpensive mold silicone infusion technique is recognized by neuroanatomical laboratories. This study improved the mold silicone staining link and explored the best mold silicone staining method.

Method

Vascular perfusion was performed in six cases of antiseptic fixed cadavers, three of which applied the conventional perfusion technique with fat-soluble dye mold silicone, and the other three applied the rapid curing technique after perfusion with water-soluble dye mold silicone.

Results

The three fat-soluble dye-stained silicone perfusion specimens showed extravasation of dye and staining of brain tissues and/or cranial nerves, which became more severe with time. Three water-soluble dye-stained silicone perfusion specimens showed good perfusion, bright colors, distinct contrast, and no staining of brain tissues and cranial nerves.

Conclusion

The technique of rapid solidification of blood vessel perfusion in cadaveric head by using water-soluble dye-stained silica gel can make the arteries and veins clearly displayed, with bright colors and sharp contrasts, and the cadaveric brain tissue and cranial nerves are not stained. It is suitable for neuroanatomy teaching and experiment.

Introduction

With the development of science and technology, neuroimaging combined with 3D printing technology and virtual reality technology has made neuroanatomical learning methods more and more diversified, but the neuroanatomical learning method based on cadaver head dissection operation is still considered the most effective neuroanatomical learning method by the majority of neurosurgeons [1]. In cadaver dissection, and especially in cerebrovascular dissection, the excellent perfusion of the blood vessels, by which the vessels are filled, and the colors of the arteries and veins are distinguished and easily recognized, has a great visual effect in the operation of cerebrovascular dissection and is conducive to teaching and experimentation, as well as to the taking of anatomical photographs.

Colored silicone perfusion is currently a common method of cadaveric vascular perfusion and is used by most research centers [2]. The silicone (3110RTV) and diluent (200fluid 5.0CS) used for it are produced by Dow Corning, USA. Because Dow Corning silicone and diluents are expensive and difficult to obtain, domestic neuroanatomy laboratories, especially for initial or microscale neuroanatomy, mostly use

molded silicone as a substitute. Hao and Wang et al. used fat-soluble dye in the process of mold silicone vascular perfusion [3–4]. In this study, we applied water-soluble dye dielectric silicone to perfuse cadaver blood vessels after a fast-curing technique and obtained favorable perfusion results with bright vessel colors, no dye percolation, and no staining of brain tissue and nerves.

Materials and Method

1. **Experimental materials and instruments**

Mold silicone (825# mold silicone, translucent, 1kg, produced by China Quanzhou City Linde Trading Co, Ltd.), thinner (silicone oil, 100ml, produced by China Quanzhou City Linde Trading Co. Ltd. of China), concentrated water-based color paste (8112 Big Red and 8151 Copper phthalocyanine, 1kg package, produced by Tong Cheng City Torchbearer Co., Ltd. of China), several plastic measuring cups (250ml) and 5 ~ 50mL syringes, transparent buckets (33cm in diameter and 30cm in height), ties and sutures, tee connectors, several homemade arteriovenous cannulate (intravenous injection exhaust tubes, indwelling needle catheters), routine anatomical Instruments; 6 formalin embalmed and fixed cadaveric head specimens > 2 years old and without any signs of cranial trauma, craniofacial deformity or previous intracranial operations (e.g., scalp incision, craniotomy or drilling), of any gender or age, were provided by the anatomy teaching and research department of Capital Medical University, where cadaveric head perfusion and dissection were performed.

2. Method

Stage1: Material Collection

The head of the cadaver was cut from the C7 level of the cervical spine with a swing saw to facilitate the placement of the V1 segment of the vertebral artery by revealing the tube and avoiding the operation of biting open the transverse foramen to expose the vertebral artery.

Stage2: Exposure to vessels and fixation

The connective tissue was first separated from the vessels, and the connective tissue of the common carotid artery on both sides was separated with scissors to find the common carotid artery on both sides, exposing at least 2–3 cm of the vessel wall, and the vertebral artery was freed in the same way, exposing at least 1.5 cm of the vessel wall (biting open the transverse foramen if necessary), and the internal jugular vein, exposing at least 2–3 cm of the vessel wall.

Stage3: Insertion and fixation of the tube

We need to apply a homemade cannula, an intravenous infusion tube for the common carotid artery or internal jugular vein, and an indwelling needle tube for the vertebral artery. First, a Nylon cable ties was

prepared and a homemade tube was inserted into the common carotid artery before the root was tied with the Nylon cable ties. Compared to common silk threads, Nylon cable ties are convenient, quick, secure, and do not protrude easily during perfusion. To avoid affecting the diameter of the vertebral artery, the vertebral artery is fixed with 2 – 0 silk thread (it is easy to close the vertebral artery with Nylon cable ties) to prevent accidental displacement, and use a thicker catheter as commonly as possible. Minimize injection resistance.

Stage4: Flushing of blood vessels

Antiseptic fixed cadaver heads have additional thrombosis in the blood vessels, particularly in the venous system, and therefore vascular flushing is critical. A 50 ml syringe of room temperature tap water was used to flush the vessel repeatedly to remove the clot and sediment, avoiding sudden pressure pushing during the flush, and gradually increasing the pressure according to the level of vascular clearance until the clot was drained. The flushing sequence is recommended to begin with the arteries and proceed to the veins: first the common carotid artery, second the vertebral artery, third the dominant internal jugular vein, and finally the non-dominant internal jugular vein. The patency of the arterial system is evaluated during flushing to understand the anatomical morphology of the circle of Willis, the presence, and patency of the anterior and posterior communicating arteries, and to predict the direction of silicone flow during perfusion. The anatomical morphology of the Willis ring is assessed as follows: if the flushing of the common carotid artery on one side causes a rapid outflow from the common carotid artery on the other side, segment A1 is bilaterally patent and the anterior communicating artery is present; If the flushing of the common carotid artery causes a rapid outflow from the vertebral artery, the ipsilateral posterior communicating artery is patent; In turn, the ipsilateral apical carotid artery is patent if the flushing of the vertebral artery causes a rapid outflow from the ipsilateral common carotid artery. Similarly, during the flushing of the vertebral arteries, the lateral vertebral arteries are pressed shut to facilitate flow into the basilar arteries and the carotid system through the posterior communicating arteries. Correspondingly, during the flushing of the venous system, each jugular vein is intermittently pressed shut to assess any potential leakage through the external jugular vein or superficial venous system. Excessive clotting is often encountered during flushing, especially in the venous system, and may be treated by injecting hydrogen peroxide into the vessel for a few moments and then flushing with water; Repeated hydrogen peroxide flushing may clear the partially blocked vessels. The flushing process is finalized with a clear stream of water, which ensures that the clot has been flushed out. At this stage, there is no fixed amount of flushing as a parameter, and it is only valid when clean water is flowing out of the lateral vessel. After adequate flushing, fluid is intermittently pumped or air is pushed into the vessels with a 50 ml syringe to drain as much fluid as possible from the vessels, and finally, the cadaveric head is placed neck down, using gravity to allow as much fluid as possible to flow out of the cadaveric vessels for molded silicone perfusion.

Stage5: Color mold silicone preparation

There are two groups of fat-soluble and water-soluble dye silicones. Mix 100ml of silicone oil to 100ml of mold silicone (transparent color) in a ratio of 1:1, add fat-soluble red and blue dye and water-soluble red and blue dye, respectively, typically 3-5ml, and stir carefully to confirm that the color is satisfactory. Add 1ml of the curing agent to the fat-soluble dye silicone, mix nicely together, and set aside. The injected solution will be entirely cured in about 7 to 12 hours. Add 1.3ml of the curing agent to the water-soluble dye silicone, mix nicely and be ready to use. The injected solution will cure sufficiently in about three hours. After the curing agent is added, the injected solution will gradually become viscous until it is fully cured. At this point, the viscosity of the infusion fluid was measured to be 15000 Pa·s. Therefore, it is recommended to perform the perfusion of the vessel as soon as possible after the configuration of the perfusion solution is completed. (Table 1)

Table 1
Ratio of silicone for fat-soluble pigment and silicone for water-soluble pigment.

		Mold silicone	Silicone oil	Pigments	Curing agent
Fat-soluble pigment group	Red	100ml	100ml	5ml	1ml
	Blue	100ml	100ml	5ml	1ml
Water-soluble pigment group	Red	100ml	100ml	5ml	1.3ml
	Blue	100ml	100ml	4ml	1.3ml

Stage6: Dyeing silicone injection

Place the head specimen in a clear bucket of water and pour running water over the broken end of the neck, leaving the perfusion catheter exposed to the water. Pump the prepared stained silicone into a 20 ml syringe, drain the air from the syringe, and inject the configured red silicone from the side with the larger diameter of the internal carotid artery, to slowly push the piston to slowly inject the liquid into the carotid artery to the contralateral internal carotid artery outflow, while maintaining the pushing pressure, close the contralateral tee valve to facilitate the flow through the posterior communicating artery to the vertebrobasilar system vessels so that the specimen is more effective. From one vertebral artery, a 10-milliliter syringe is pushed at a uniform constant pressure until the lateral vertebral artery flows out, closing the lateral valve. From one side of the open vertebral artery continue to push silicone at a constant speed, at this time can feel the hand pressure conduction resistance, moderate push injection, and visible internal carotid artery channel with silicone slowly outflows, continue to maintain a modest amount of pushing configuration silicone liquid after closing the internal carotid channel. The opposite direction is operated in the same way. After perfusion of the arteries to perform internal jugular perfusion, perfusion from the dominant side of the internal jugular must be based on slowly pushing the injection against the resistance of the vein, without sudden pressure, to avoid rupture of the vein due to its thin wall. Because silicone is water-soluble, if it leaks, the specimen surface will form a mass floating in the water, so the leak can be clamped in time with a vascular clamp, and the leaking silicone can be retrieved afterward.

Stage7: Specimen preservation

After perfusion, cadaveric specimens are placed head down in a cool place and stored in 75% medical alcohol after the curing of the perfusate is completed (although curing can be completed after 3 to 12 hours, for simplification of operation our laboratory mainly the next day after perfusion).

Stage8: Anatomical observation of perfused specimens

Specimens were observed anatomically from 1 week to 2 months after perfusion to observe vessel filling, vessel staining, brain tissue staining, and brain tissue staining by perfusate leakage from ruptured vessels.

Results

Specimens were dissected and observed for vascular staining and intracranial tissue staining after perfusion was completed.

Specimen 1 was perfused with fat-soluble dye silicone for arterial perfusion, and a post-mortem examination was performed 1 week after perfusion to look at the vertebral arteries, the Willis ring at the base of the skull, and the surrounding vessels filled with the stained silicone, with lightly stained perivascular tissue (Fig. 1).

Specimen 2, arteries and veins were perfused with fat-soluble dye silicone and an autopsy was performed 1.5 months after the perfusion was completed. The cortical arteries were filled with stained silicone, the superior sagittal sinuses and superior drain veins were filled with stained silicone, and the cerebral cortex was heavily stained (Fig. 2).

Specimen 3 was perfused with silicone in a fat-soluble dye, and the internal carotid, anterior, and middle cerebral arteries were filled with the stained silicone for anatomical observation 2 weeks after perfusion, and the cranial nerves were severely stained (Fig. 3).

Specimen 4, the artery was perfused with water-soluble dye silicone and a post-mortem examination was performed 1 week after perfusion. The cerebral and cerebellar arteries were filled with stained silicone, and the right posterior communicating artery ruptured, causing a silicone leak, but the brain tissue was not stained (Fig. 4).

Specimen 5 was stained with a water-soluble dye to stain the silica-perfused veins. After 2 weeks of perfusion, the basilar vein was stained and filled with silicone in a bright color, the right basilar vein was ruptured, causing a silicone leak, and the cerebral cortex was lightly stained. (Fig. 5)

Specimen 6 was perfused with water-soluble dye silicone in the arteries and veins, and anatomical observations were performed 2 months after perfusion; the right temporal and occipital cortical arteries

were stained and filled with silicone, the right transverse sinus and Labbe vein were stained and filled with silicone, and no brain tissue was stained (Fig. 6).

Perfusion specimens of fat-soluble dye silicone have all shown staining of brain tissue and cranial nerves due to dye exudation, with staining becoming more severe over time. The water-soluble dye-stained silicone perfusion specimens showed no staining of brain tissues and cranial nerves, and even if the blood vessels ruptured during the perfusion process, resulting in direct contact between the leaking silicone and brain tissues, only mild staining of the brain cortex in contact with the silicone was caused. (Table 2)

Table 2
Comparison of results between silicone with fat-soluble pigment group and silicone with water-soluble pigment group

Pigments	Specimens	Arteries	Veins	Perfusion anatomical interval	Vascular staining	Brain tissue and nerve staining
Fat-soluble pigment group	1	i/a	N/A	1 week	Satisfaction	Mild
	2	i/a	i/a	6 weeks	Satisfaction	Severe
	3	i/a	N/A	2 weeks	Satisfaction	Severe
Water-soluble pigment group	4	i/a	N/A	1 week	Very satisfied	None
	5	N/A	i/a	2 weeks	Very satisfied	Mild
	6	i/a	i/a	8 weeks	Very satisfied	None

Discussion

With the rapid development of microsurgery, neurosurgeons have become more demanding in their knowledge of neuroanatomy. It provides security to create modern surgical modalities in neurosurgery. Currently, the anatomical study of cadaver head specimens is the best way to practice surgical modalities and learn anatomy outside the operating room. Pre-dissection preparation of cadaveric head specimens improves anatomy and is more conducive to our accurate understanding of the vascular and nervous systems [5].

Intravenous injection of different colored perfusates is a traditional anatomical technique used primarily in the preparation of head and neck specimens for intracranial and extracranial vascular dissection. The original injection techniques relied on colored materials that were liquid when heated and solidified when cooled, such as wax and paint mixtures, and these techniques were called "thermal injection". These techniques were called "hot injections". "Hot injections" were used as early as the 17th century and were

gradually replaced by "cold injections", in which a catalyst was mixed with the perfusion solution so that it would solidify after the perfusion.

Formalin has proven to be a major advance in embalming science, with excellent antiseptic properties, outstanding antibacterial properties, low price, and easy access, and has become the standard embalming fluid for human autopsy studies. Formalin embalmed cadaver specimens were barely moldy at the time of the autopsy. However, formalin has an irritating odor and stiffens tissues, and prolonged inhalation and exposure may pose a health risk. For these reasons, recent efforts have been made to find better tissue preservatives. In our anatomy laboratory, 75% alcohol is commonly used as a substitute, which can slightly improve the stiffness of formalin-fixed specimens and significantly reduce the irritating odor of formalin solution, and still has an excellent preservative effect, and some specimens in our laboratory have been preserved in 75% alcohol for up to 2 years without mold and additional spoilage.

Most neuroanatomy laboratories in China currently use preserved fixed cadaver heads for anatomy teaching or experiments. In some cadavers, the intravascular thrombus is so large and strong that it exclusively clogs the blood vessels, and repeated flushes fail to clear them, especially in the venous system. The choice of arterial and venous perfusion in the six specimens was determined by the patency of the flush.

Mold silicone and silicone oil are fat-soluble and usually apply oil-based dye both fat-soluble dye staining, whether recommended by mold silicone distributors, or domestic Hao and Wang et al. used fat-soluble dye line mold silicone staining during mold silicone vascular perfusion [3–4]. It is well known that due to the alteration of the postmortem vascular system, the permeability of the vessel wall is significantly increased (Fig. 7) and there is a possibility of infiltration of the perfusate injected in the lumen of the vessel into the surrounding tissue [6]. This effect has even been exploited during embalming, where an increase in soft tissue swelling indicates successful embalming [7]. Coupled with the fact that brain tissue is abundant in lipid components, fat-soluble dye are more likely than water-soluble dye to penetrate the vessel walls, and the increased permeability leads to dye penetration into the stained brain tissue. This may be the reason why the three specimens perfused with silicone from the lipid-soluble dye stain molds in this study showed different levels of brain tissue stain. Although the water-soluble dye were not dissolved in the silicone oil mixture, after uniform mixing, the water-soluble dye could be uniformly suspended in the molded silicone perfusion solution, and the curing time of silicone was shortened by using the dose of curing agent, and the rapid curing of silicone caused the water-soluble dye to be permanently dispersed in the cured molded silicone and had the effect of uniformly staining the silicone.

The flowability of silicone is not good, too rapid curing will affect the flow of silicone to the finer distal vessels, in the continuous exploration, the members of this research team agreed that curing about 3 hours after injection is conducive to the filling of the distal vessels with silicone while ensuring that the water-soluble dye still maintain uniform dispersion.

The ratio of silicone to silicone oil is proportional to the flow rate of the final injection and inversely proportional to the ductility of the cured silicone. Rhoton and colleagues recommended a silicone to silicone oil ratio of 1:1 to 1:2 [2], but the material used was the silicone from Dow Corning mentioned in the previous paper, not the mold silicone commonly used in domestic anatomy laboratories. As a result of previous experience with mold silicone injection in this anatomical laboratory, the mold silicone was configured with a 1:1 ratio of mold silicone to silicone oil, resulting in better fluidity and some ductility after curing. Finally, a ratio of silicone: silicone oil: curing agent = 100ml: 100ml: 1.3ml was used to configure the infused silicone to achieve satisfactory results.

In addition, silicone is fat-soluble and insoluble in water, taking advantage of this physical property, cadaveric specimens are placed in water for perfusion, while for easy observation this operation is performed in a transparent bucket of water, and silicone pieces found to leak out of the water can be easily retrieved to reduce the contamination of specimens by leaking silicone from blood vessels and dropping silicone during perfusion.

Little literature has been devoted to cadaveric perfusion techniques, and disagreement persists as to the best ones. In 1999, Sanan and his colleagues [2] described their technique for performing body silicone injections at the University of Cincinnati. In a review that followed that report, Day and Rhoton also described their technique, which was not substantially different from that of Sanan et al. and also used silicone. Also, the choice of grout materials is varied, with latex, gelatin, and silicone. Due to the difficulty in procuring silicone materials commonly used in foreign anatomical laboratories in China, most domestic anatomical laboratories have studied the application of molded silicone for perfusion and obtained better perfusion results. Our study concluded that water-soluble dye has more vivid colors than fat-soluble dye, that vascular staining results in superior visual results, and that the images taken are more aesthetically pleasing and more conducive to teaching and experimentation. We hope that this study will play a role in establishing standardized procedures for the perfusion of cadaver vessels in domestic neuroanatomy laboratories.

Conclusion

We applied water-soluble dye to stain the molded silicone perfusion solution and precisely formulated the ratio of molded silicone, silicone oil, and the curing agent, which resulted in rapid curing of the perfusion solution and vivid and contrasting vascular staining of the perfused specimens, with no staining of the brain tissue. This method has the advantage of being simple to operate, inexpensive, and easy to purchase application materials, and the dissection operation can be performed in a short time after the rapid curing of the perfusion solution. The favorable perfusion effect improves the quality of dissection of cadaver heads and establishes a solid foundation for teaching and experimentation in advanced neuroanatomy. In the meantime, we describe additional details of molded silicone vascular perfusion for dissection beginners and beginning neuroanatomy students to learn and discuss together.

Declarations

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contribution

P.and H. wrote the main manuscript text and H.prepared figures 1-7. All authors reviewed the manuscript.

Acknowledgment

The authors sincerely thank those who donated their bodies to science so that anatomical research could be performed. Results from such research can potentially increase mankind's overall knowledge that can then improve patient care. Therefore, these donors and their families deserve our highest gratitude.

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Figures

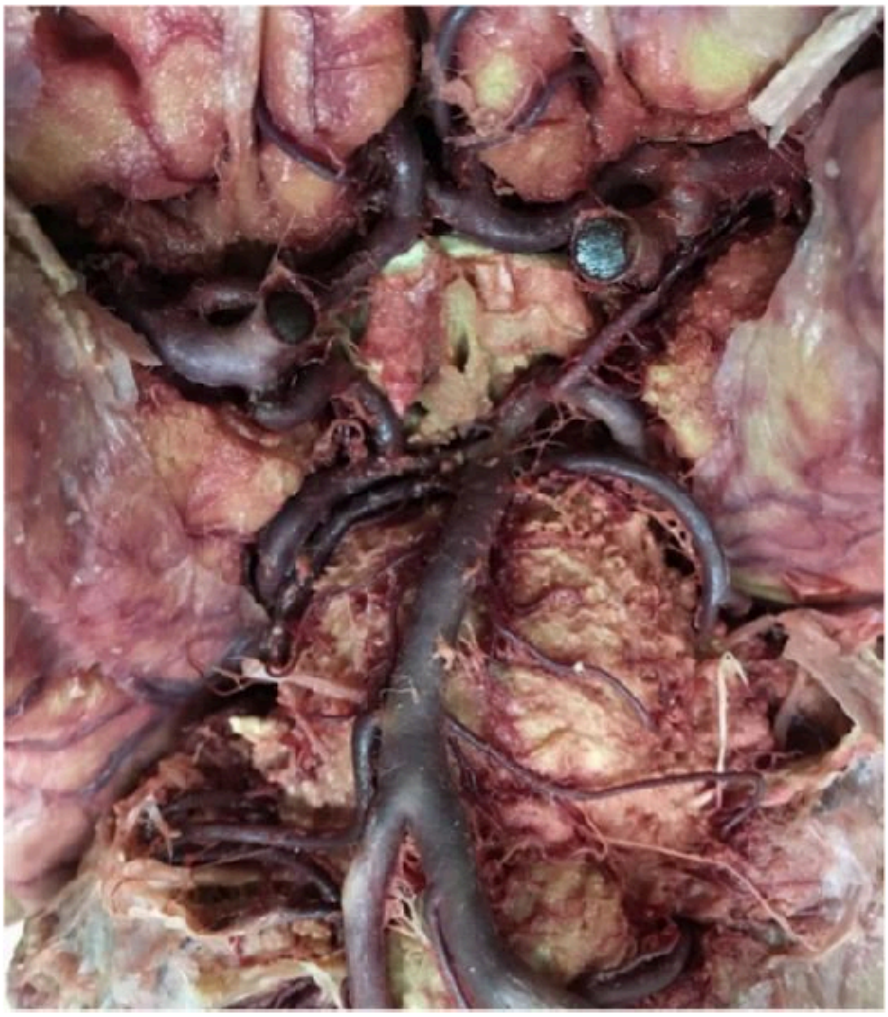


Fig 1 Lipid-soluble pigment staining effect (short-term) 1 week after arterial perfusion of specimen No. 1, lightly stained.

Figure 1

See image above for figure legend.



Fig 2 Lipid-soluble pigment staining effect (long-term) After 6 weeks of arterial and venous perfusion of specimen No.2 , heavy staining.Large areas of the brain surface were stained an abnormal pink color, even the facial muscles.

Figure 2

See image above for figure legend.

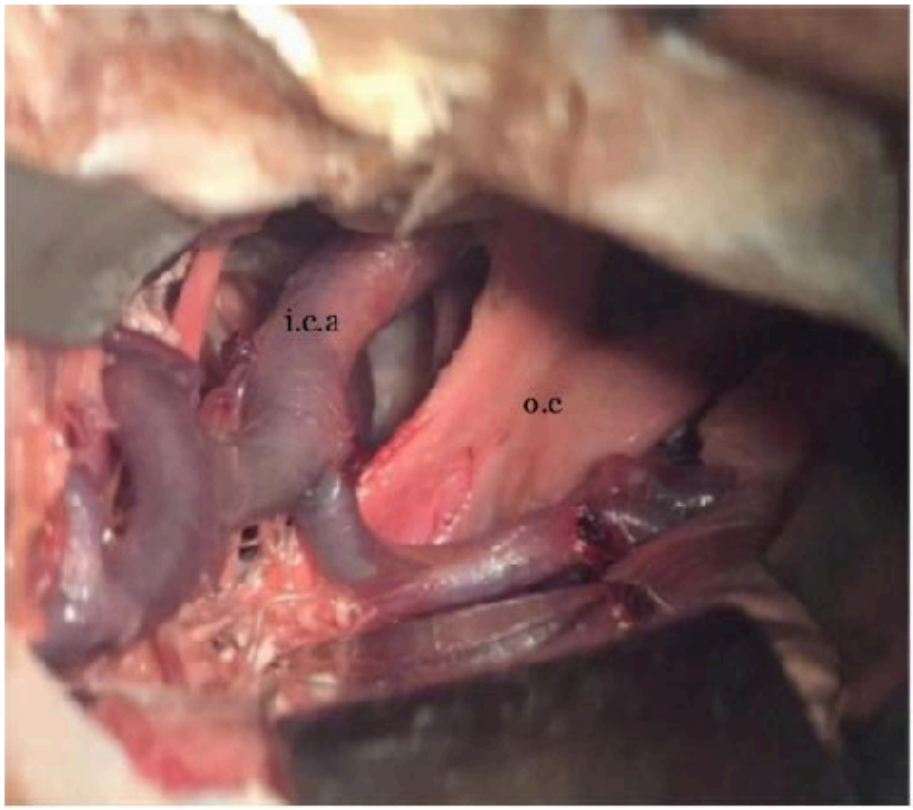


Fig 3 Lipid-soluble pigment staining effect (short-term) 2 week after arterial perfusion of specimen No. 3, heavy staining.o.c:optic chiasm,i.c.a:internal carotid artery

Figure 3

See image above for figure legend.



Fig 4 Water-soluble pigment staining effect (short-term) 1 week after arterial perfusion of specimen No. 4, no staining.

Figure 4

See image above for figure legend.



Fig 5 Water-soluble pigment staining effect (short-term) 2 week after venous perfusion of specimen No. 5, lightly stained.

Figure 5

See image above for figure legend.



Fig 6 Water-soluble pigment staining effect (long-term) 8 week after arterial and venous perfusion of specimen No. 6, no staining.

Figure 6

See image above for figure legend.

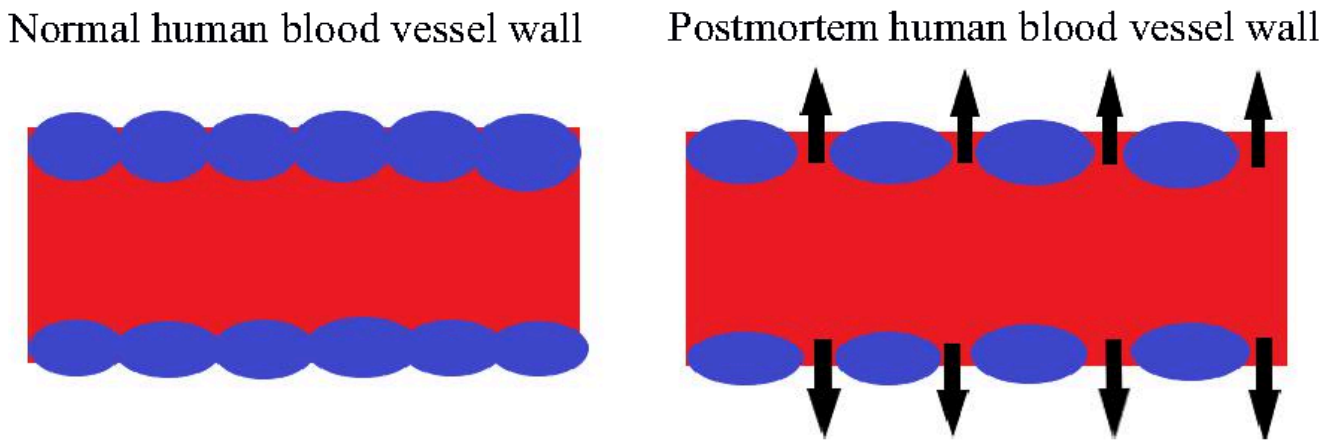


Fig 7 Vascular variation pattern diagram Vascular walls become porous after death, especially changes in the endothelium, leading to increased vascular permeability prone to leakage.

Figure 7

See image above for figure legend.

Supplementary Files

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