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Original Investigation

A Very Quickly Prepared, Colored Silicone Material for Injecting into Cerebral Vasculature for Anatomical Dissection: A Novel and Suitable Material for both Fresh and Non-Fresh Cadavers

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ABSTRACT

AIM: Cadaveric studies have a great impact on neuroanatomy learning. Cadaver preparation may take a lot of effort, especially at the phase of intravascular color filling. The authors describe their silicone dye technique and a novel mixture which is self-curing, quick to prepare and easy to inject.

MATERIAL and METHODS: The first one of these processes is undoubtedly embalming and decapitation of the cadaver. If possible, the most appropriate time that should be preferred is immediately after the donor's death. Preparation for cadaveric dissection of the brain requires some fundamental steps that can be summarized into:

a) Embalming and decapitation, b) Exposing, cannulization and irrigation of main vascular structures, c) Preparing colored silicone, d) Injection of colored silicone and staining the vascular tree, e) Sample maintenance

RESULTS: Our method of preparation of silicone dye and injection enables neurosurgeons and anatomists to fill cerebral and dural vascular structures, and even diploic veins nicely in both fresh and aged cadaveric heads. Moreover, the main vascular structures and their branches in the lateral and third ventricles are painted remarkably beautifully.

CONCLUSION: We tried to provide our experience about the preparation of head cadavers for anatomical dissection using a novel mixture of colored silicone that is very easy to prepare and inject with very satisfactory results.

KEYWORDS: Head cadaver, Silicone dye, Cerebral vasculature, Anatomy, Dissection

INTRODUCTION

There is no doubt that extensive knowledge of neuroanatomy plays a vital role in neurosurgery practice. Cadaveric studies have a great impact on neuroanatomy learning. Cadaver preparation may take a lot of effort, especially at the phase of intravascular color filling. Preparation for cadaveric dissection of the brain requires some fundamental steps that can be summarized into:

a) Embalming and decapitation, b) Exposing, cannulization and irrigation of the main vascular structures, c) Preparing colored silicone, d) Injection of colored silicone and staining the vascular tree, and finally e) Sample maintenance. Each of these steps is closely related to one another and must be executed in a stepwise fashion in order to have a satisfactory result. There are several studies, which describe these steps, in more or less the same way (1-4).

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MATERIAL and METHODS

Embalming and Decapitation

The first one of these processes is undoubtedly embalming and decapitation of the cadaver. If possible, the most appropriate time that should be preferred is immediately after donor's death. However, this might not be always possible to perform. Therefore, the most common practice is the one performed within the first 20-40 hours following decease.

The head must be embalmed and fixed before the decapitation phase. The most suitable material to be used in this phase is 10% formaldehyde solution. Along with providing long-term structural preservation of the cadaver, formaldehyde also denatures microorganisms including human immunodeficiency virus (HIV), slow viruses etc. and prevents them from reproducing in the tissue. Formaldehyde also fixes and hardens brain tissue and performing dissection on non-fixed brain tissue is not possible as the tissue is very soft and easily dispersible. The most significant disadvantage of formaldehyde is its irritating odor and its ability to harden the tissue to a great extent under long-term exposure. Solutions recommended to eliminate this issue shall be mentioned below.

Next, both common carotid arteries, vertebral arteries, and internal jugular veins should be percutaneously cannulized and thoroughly rinsed with this solution. This procedure must be continued for at least thirty minutes at each instance for about three days.

The decapitation procedure must be performed after treating with formaldehyde. The decapitation procedure is performed with the help of surgical blades and oscillating saws. The most important factor to be taken into account at this point is to perform the procedure on the lowest section of the neck as far as possible. Decapitation performed at the fourth (C4) or fifth (C5) cervical vertebra level enables sufficiently long extra-cranial veins to be accessible and this provides great convenience during cannulization of such veins.

Staining Vascular Structures

Properly staining vascular structures in the intracranial space facilitates cadaver use. Making vascular anatomy, which is an integral part of all neurosurgical practices, more understandable is possible through staining vascular structures. Thus, neurosurgeons orientate themselves with the underlying anatomical structures more easily and are able to improve their dissection abilities. Staining vascular structures consists of a series of phases, each one of which shall be individually addressed below and should be taken into account. Although plenty of anatomical dissection studies are available in the literature, studies that present detailed technical information, particularly regarding the staining procedure and the earlier preparation process, are rather few in number (2,4).

Exposing Main Veins

At this stage, six vessels in total located in the neck (two common carotid arteries, two vertebral arteries, and two internal jugular veins) should be revealed. Ease of operation can be provided by fixing the head. A rigid fixation system is not required for this; any method that can constrain the head in the upside-down position is sufficient. Custom-built square and rectangular shaped marble blocks of varying thickness and height are used in our neuroanatomy laboratory where this study was performed and the results are quite satisfactory.

Each one of the vessels must be revealed by means of sharp and obtuse dissection, and a minimum two to three centimeters of vein length should be obtained. Use of bone rongeurs or electrical and/or pneumatic drill bits might be required for the purpose of extending transverse foramina, especially when revealing vertebral arteries. Moreover, microscope use is encouraged during this procedure in our neuroanatomy laboratory as it is believed that microscope use provides great convenience during the procedure and also contributes to improving microscope-using skills. Basic supplies required for silicone injection are shown in Table I.

Cannulization of Main Veins

Each one of the vessels must be cannulized with plastic tubes of appropriate width at this stage. Although cannulization can be performed using any type of tube, suction and/or nasogastric tubes are preferred in our neuroanatomy laboratory with regards to both the abundant availability of sizes with varying width and the costs. The most significant point at this stage is choosing suitable sutures and steady placement of tubes to the vessels since injecting the dye into vessels requires injection with a certain force. If the tube protrudes out of the vein during the injection procedure, this proves to be extremely frustrating and disappointing (Figure 1A).

Irrigation of Main Arteries and Veins

The most important stage that determines the outcome of quality staining of vascular structures is the irrigation of cannulized vessels. The purpose is to remove the blood clots and formaldehyde in the vessels. Satisfactory staining should not be expected in the vessels where adequate irrigation was not applied. Using tap water is sufficient for irrigation.

Table I: Basic Supplies Required for Silicone Injection

Graduated cylinder
Bone rongeur
Tissue forceps (assorted sizes)
Scissors
Bone wax
0, 2-0 and 3-0 sutures (many)
Plastic tubing (assorted sizes)
60-ml syringes (at least four per cadaver)
Vessel clamps (at least six per cadaver)
Blue liquid paint
Red liquid paint
Silicone

Each one of the veins should be irrigated individually with a gavage injector (60 centiliters) using plenty of tap water. Another advantage of irrigation is that it can give an idea to the researcher about whether the Willis polygon is clear and open. Water coming out of particularly the contralateral artery or from different arteries during the irrigation phase of arteries is acknowledged as the best indicator of an un-obstructed system. Each laboratory may determine its own irrigation duration. The important thing is to assure that the water coming out of the contralateral vessel is clear. It was observed that two-day irrigation, each lasting half an hour is sufficient. Another important point at this stage is sealing vascular structures which cause leaks that may originate from the paraspinal muscles or subcutaneous tissue by means of using clamps or suitable sutures. Moreover, it is also required to seal the spinal tract with bone wax or a sponge. Thus, it is possible to prevent the dye coming out of leakage points instead of intracranial veins during staining. However, some laboratories may choose not to plug the spinal canal since they believe that plugging could make the silicone injection more difficult, which we did not experience so far.

Staining Main Veins

It is a known fact that many supplementary materials such as resin, latex, India ink and silicone are utilized for staining vascular structures. Based on our experience, however, a colored, 100% silicone achieves the best results: we experienced that it can preserve anatomical features of vascular structures better than any other material.

Preparing colored silicone dye

Information about the preparation of colored silicone dye in the literature is extremely limited. Still, the impression acquired from both the literature and correspondences made with overseas laboratories by our neuroanatomy laboratory shows that colored silicone dye is prepared by mixing different materials. Utilizing silicone, red or blue powder or liquid paint, thinner and a catalyst is the common practice in almost all. These materials are mixed with varying amounts and later followed by injection. A completely different, yet a lot more easily prepared protocol is followed in our neuroanatomy laboratory. This protocol contains two silicone components sufficient to be mixed on a one-to-one ratio which are defined as A and B (ELASTOSIL P 7670 A and ELASTOSIL P 7670 B; Wacker Silicones, Munich, Germany) with adequate viscosity (2000 mPa.s; 1.00 g/cm^3 at 20^{o}C), and being able to be vulcanized in a period less than an hour without any need for an additional catalyst, and red – blue liquid paint compatible with these components and sufficient to be added only at a ratio of one percent (ELASTOSIL Colour Paste FL Dark Blue RAL 5010 and ELASTOSIL Colour Paste FL Dark Red RAL 3000; Wacker Silicones, Munich, Germany). Materials required for preparation of the colored silicone mixture is shown in Table II.

Another significant characteristic of this mixture is that the mixture does not dilate and shrivel during vulcanization. Therefore, anatomical features of vascular structures can be preserved. If it is desired to keep the period between staining the cadavers and initiating dissection short, vulcanization can be acquired in an even shorter duration (5 to 10 minutes) by adding a catalyst procured from the same company (Catalyst EP, Wacker Silicones, Munich, Germany) to the total amount of mixture at a ratio of 0.1%-0.2% and it is then possible to start dissection within a maximum of 1-2 hours. The saturation and brightness of the colors from this mixture are extremely satisfactory.

Injection of colored silicone mixture

Approximately 100 ml mixture for staining all arterial trees and approximately 150 ml mixture for staining venous trees will suffice. The injection is performed by utilizing 60-ml gavage injectors. At this stage, surgical clamps must be kept ready and available in an easily accessible location. A clamp must hold each vessel before the procedure. Regardless of the viscosity of the mixture, a considerable resistance is experienced during

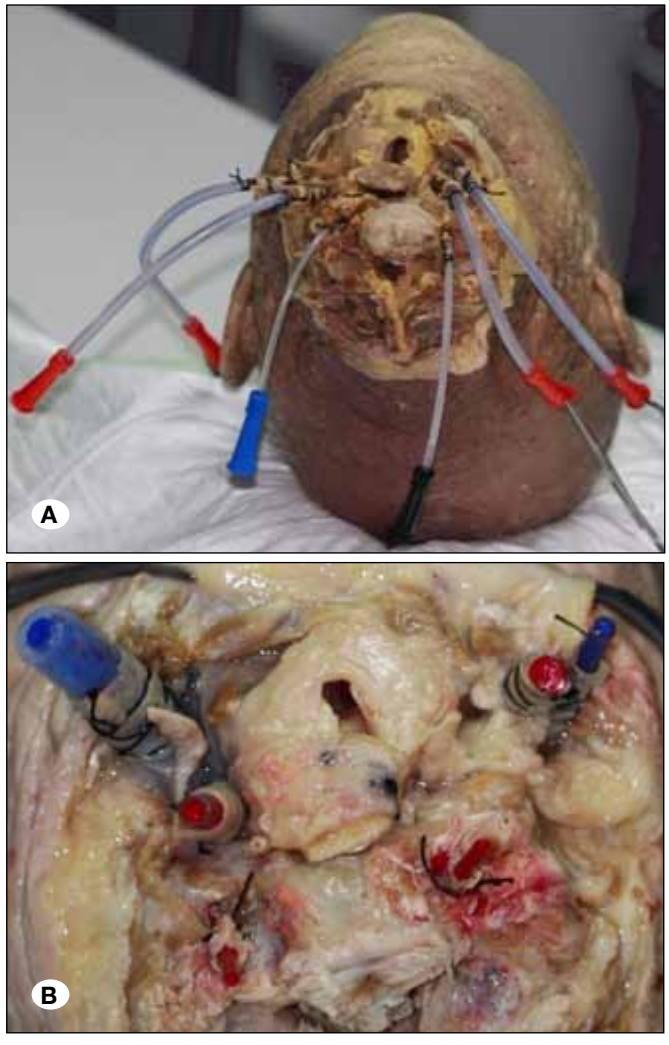

Figure 1: A) The main vessels of the neck have been isolated and dissected from the surrounding tissues. The foramen transversaria need to be enlarged to expose and cannulate the vertebral arteries. **B)** Jugular veins, common carotid arteries and vertebral arteries are cannulated with appropriately sized tubing. The cannulae should be secured very tightly since dislodgement is annoying.

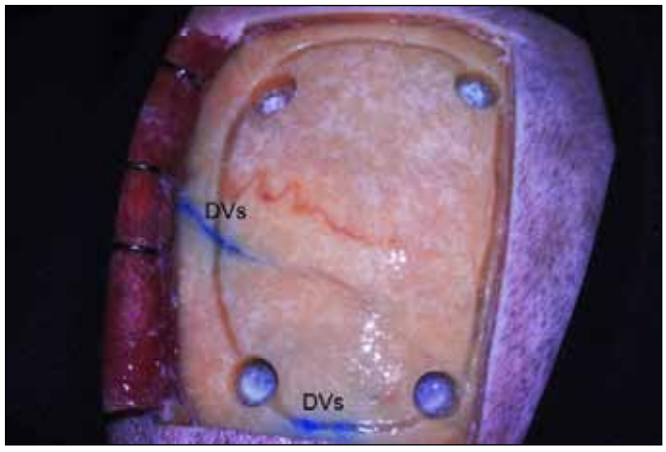

Figure 2: The bone flap of the injected specimen. The diploic veins (DVs) within the bone flap are nicely depicted.

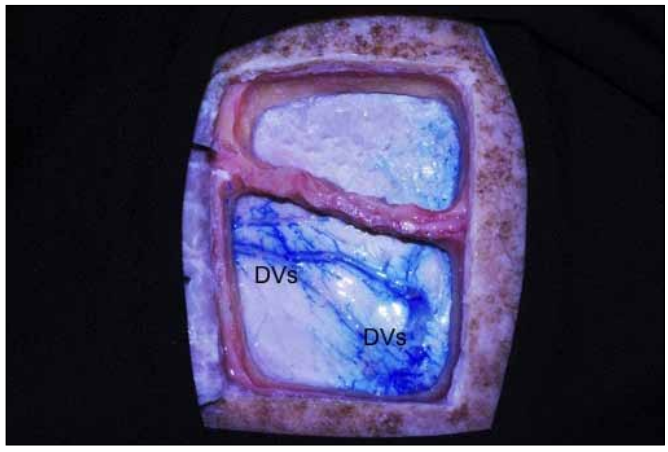

Figure 3: The dura of the injected specimen. The dura is preserved and dural veins (DVs) are visualized clearly by this injection technique.

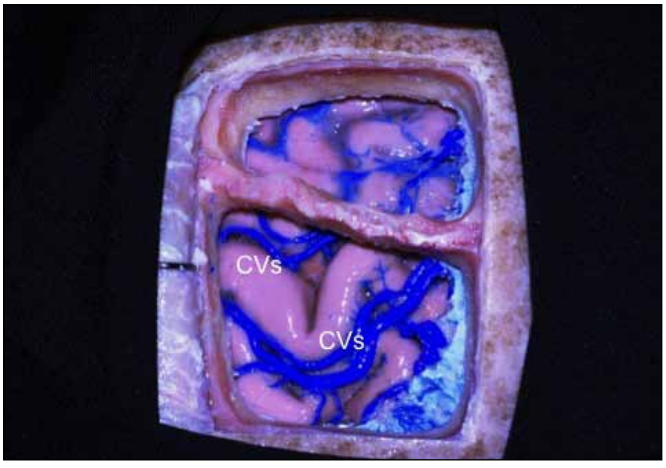

Figure 4: Cortical veins in the injected specimen. The dura has been removed and cortical veins (CVs) are exposed. The veins are seen beautifully visualized this injection technique.

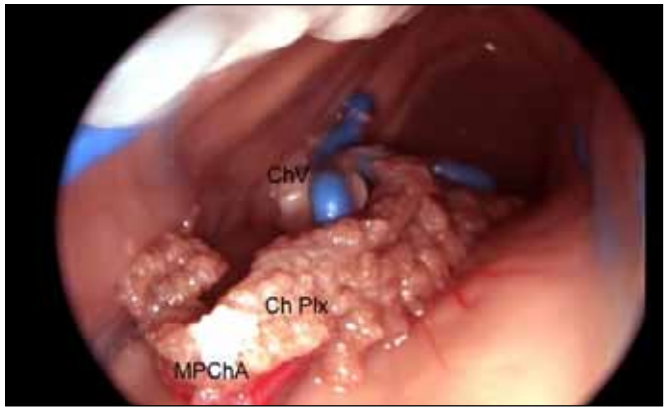

Figure 5: Neuroendoscopic view of the lateral ventricle of the injected specimen. Choroid plexus (ChPlx), choroidal veins, medial posterior choroidal artery (MPChA) and its tiny branches are well visualized.

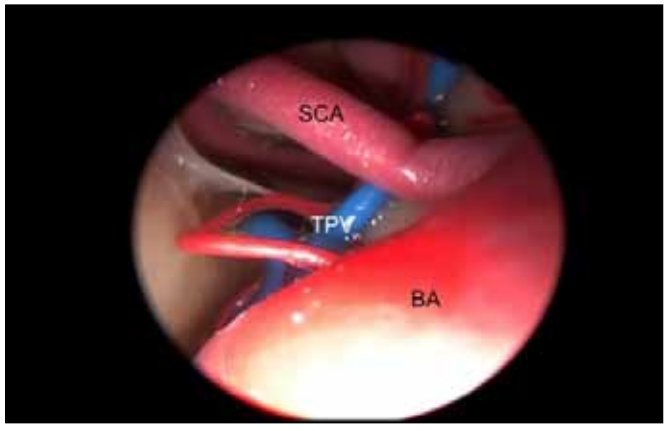

Figure 6: Neuroendoscopic view of the third ventricle of the injected specimen. Tip of basilar artery (BA), right superior cerebellar artery (SCA), and vein which is considered to be the transverse pontine vein (TPV) are clearly seen in the prepontine cistern.

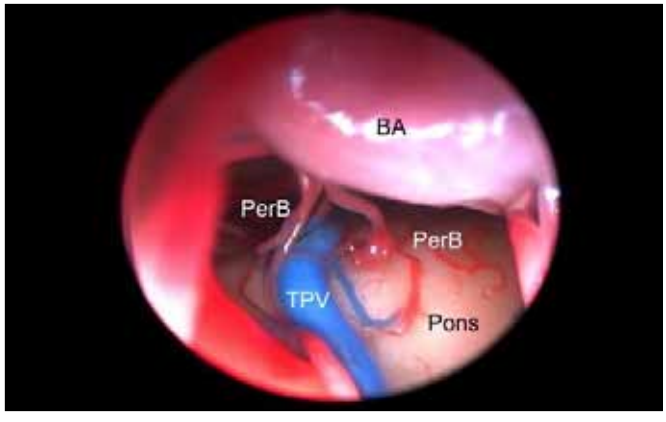

Figure 7: Another neuroendoscopic view of the third ventricle of the same injected specimen. Another view of the prepontine cistern of the same injected specimen. Basilar artery (BA) and its perforating branches (Per B) into the pons can be seen. The venous structure stretching on the pons from among the perforating arteries is again considered to be transverse pontine vein (TPV). All these structures are injected remarkably with this technique.

Table II: Materials Required for Preparation of Colored Silicone Mixture

Silicone Mixture	Sample Preparation per One Head
Red	
Red liquid dye	1/2 teaspoon
Silicone component A	50 ml
Silicone component B	50 ml
Catalyst (if needed)	0.1% of mixture volume
Blue	
Blue liquid dye	1/2 teaspoon
Silicone component A	75 ml
Silicone component B	75 ml
Catalyst (if needed)	0.1% of mixture volume

the procedure. Therefore, the injection must be performed by at least two people. Readily available silicon guns provide great convenience during this phase. Injections should be initiated at first on the internal jugular vein of one side. When the blue mixture reaches the opposing internal jugular vein, the vein should be held by a clamp and the mixture should be injected to the vein on the other side. Then, both veins should be held by a clamp. Injection of the red mixture shall be initiated on one of the common carotid arteries in the same manner. When the mixture is seen coming from the opposing common carotid artery, the initial artery should be held by a clamp and application of the mixture should be continued from the opposing artery. Then, both arteries should be held by a clamp. The same procedure should be repeated for both vertebral arteries in turn.

This procedure requires application of force. However, patience must be kept during injection and excessive use of force should be avoided. Otherwise, the tubes may protrude from the vessels and the quality of cadavers can be negatively affected for use if intracranial veins are punctured and the dye mixture flows out of the vessels. It must be taken into account that the dye mixture can flow out of the open-ended vessels under the skin and in the paraspinal muscles during injection and an attempt must be made to obstruct such vessels by a clamp or a suture (Figure 1B). This phase is intrinsically very messy. Regardless of any action, the silicone mixture overflows and contaminates the outer section of the cadaver as well. Another advantage of the above-mentioned protocol is that this silicone can be easily cleaned from any surface with a wet sponge.

Sample Maintenance

Curing process of the applied dye mixture varies depending on the materials used. While this duration is shorter than any other protocol in the literature, it is deemed appropriate to wait for at least several hours.

There are many different approaches regarding the storage conditions of the cadavers. Formaldehyde use has been acknowledged as the standard for many years. However, as mentioned above, cadavers exposed to formaldehyde for a long duration become extremely rigid and therefore retraction of brain tissue becomes difficult to the extent of not even allowing attenuation. Therefore, the use of 66% ethanol is recommended. Not altering the magnitude of brain tissue, preserving the tissue consistency prior to alcohol and maintaining the dye mixture are the advantages of alcohol. However, we began to preserve our head cadavers, wrapping them with suitable coverings in the refrigerator under -10°C to -20°C if they were not to be used in a short time, which became ready to be used again after keeping at 1-2°C overnight. The result is quite satisfactory.

■ DISCUSSION

Preparation of head cadavers for anatomical dissection is time-consuming, troublesome and sometimes annoying. However, it is an exciting opportunity for educational purposes for both junior and senior neurosurgeons (2-4). Anatomical specimens such as head cadavers may not be difficult to obtain, but a satisfactory vascular staining is not easy during initial attempts of the preparing and injecting process.

Using a combination of silicone, red or blue powder paint, thinner and a catalyst is the common practice in almost all laboratories. These materials are mixed with varying amounts and later followed by injection (2-4). However, both the silicone and dye used in our study are in liquid form and a thinner solution is not required for preparation. In addition, curing of this mixture requires less than half an hour even without adding a catalyst.

We used ten non-fresh, aged head cadavers in this study. We believe that our method of preparation of silicone dye and injection enables neurosurgeons and anatomists to fill cerebral and dural vascular structures, and even diploic veins nicely in both fresh and also aged cadaveric heads (Figures 2-4). Moreover, main vascular structures and their branches in lateral and third ventricles are painted remarkably beautifully (Figures 5-7).

To conclude, we tried to provide our experience about the preparation of head cadavers for anatomical dissection using a novel mixture of colored silicone that is very easy to prepare and inject with very satisfactory results.

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