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# The Six-Point Injection Technique: A Non-Immersion Method for Enhancing Cadaveric Tissue Quality

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## Abstract

Two-point injections of embalming fluid without venous drainage, along with immersion in a post-fixative pool, has been used for several decades in the Department of Anatomy, Chiang Mai University (CMU). However, tissue decomposition was frequently observed during dissection. To address this, a Modified Embalming Method (MEM), utilizing a six-point injection technique with venous drainage, was tested. This study evaluates the effectiveness of MEM compared to the traditional two-point method with immersion. Ten cadavers were preserved and assessed for range of motion (ROM), histological integrity, and dissection quality. The cadavers were divided into two groups: five were embalmed using MEM and stored in plastic bags at room temperature, while the other five were preserved using the Present Embalming Method (PEM), which involved two-point injection without venous drainage followed by immersion. Both groups received the same embalming fluid. After one year, ROM was measured, and dissection quality was evaluated by ten dissectors. The MEM group showed greater joint mobility and superior tissue quality for both gross anatomical and histological analysis. The enhanced perfusion achieved by MEM ensured uniform distribution of fixative throughout the body. Furthermore, MEM eliminated the need for immersion, reduced chemical use, and allowed safe storage of cadavers in mortuary bags at room temperature.

***Keywords***: *cadaver; embalming; femoral; formaldehyde; perfusion; Modified Embalming Method; MEM*

## 1. Introduction

Arterial perfusion of embalming fluids has been performed since 1638 by Frederik Ruysch. This practice has evolved over several decades (Bradbury, & Hoshino, 1978; Barton et al., 2009; Hammer et al., 2012; Naketar, & Desousa, 2012; de Varrimento, 2013; Brenner, 2014). The embalming fluids used in this procedure have also been developed to improve cadaveric quality (Kaliappan et al., 2023). In the human body, many arteries and veins are present in appropriate regions for injecting embalming fluid and draining blood from cadavers. Previous studies have introduced various injection techniques that primarily involve injecting the embalming fluid into the femoral and common carotid arteries (Batra et al., 2010; Eisma, & Wilkinson, 2014; Theeuwes et al., 2017). In general, injection methods are categorized into 5 types (Mayer, 2011; Ajileye et al., 2018): the single point, the split, the restricted cervical, the multipoint, and the six- point injections in which venous drainage methods are used.

After using various embalming fluid injection processes, some researchers preserved the cadavers by maintaining moisture in different ways, such as immersing them in an embalming fluid pool (Thiel, 1992; 2002; Kerckaert et al., 2008; Bertone et al., 2011; Eisma et al., 2013; Balta et al., 2015a; Healy et al., 2015; Hammer et al., 2015). Cadavers were stored

in plastic bags and kept in freezers (Anderson, 2006; Mills, 2010). Hayashi et al., (2014) and Sugata et al., (2016) demonstrated that cadavers can be placed in mortuary bags at room temperature. Non-immersing cadavers preserved with saturated salt solution had their tissue qualities similar to those of living humans (Durongphan et al., 2022). Our department uses a method in which cadavers are immersed in an embalming fluid pool containing formaldehyde and an 85% phenol mixture. These chemicals emit a strong odor that can disturb neighboring organizations, and also promote mold growth inside the pool, which could adversely affect employee health. In addition to health concerns, these chemicals must be regularly disposed of from the pool, which is costly.

There are several factors that delay the embalming process in the Department of Anatomy at Chiang Mai University (CMU). In accordance with Buddhist cultural practices, the funeral home is commonly used for one to three days after death. The distance between the funeral home and the department ranges from a few to two hundred kilometers. Thai law does not permit technicians to inject embalming solutions immediately after death. The recommended practice for these conditions is that cadavers should be placed in a cooled coffin until the embalming process. Although cold temperatures slow decomposition in cadavers, the bodies still lose water and develop blood clots which obstruct fluid injection (Mayer, 2011). In addition to blood cell clogging, pathogens from respiratory and digestive tracts-invade blood vessels within minutes after death and these microorganisms accelerate cadaver putrefaction. Blood has high moisture content and serves as a rich nutrient source promoting microbial growth, particularly anaerobic organisms (Burn, 1934; Kellerman et al., 1976; Heimesaat et al., 2012; Riedel, 2014; Alsharif et al., 2017).

The embalming procedure at the Department of Anatomy, Faculty of Medicine, CMU, Thailand, involves injection into both femoral arteries without blood drainage However, blood cells within the vessels can obstruct the circulation of embalming fluid. Based on our experience, embalming fluid often fails to reach the distal arms, brain, and spinal cord. Technicians usually address this issue by using a syringe to inject embalming fluid directly into the unreachable area. Instead of entering the blood vessels, the fluid remains only at the injection sites, leaving some deep tissues insufficiently embalmed. These conditions lead to decomposition and a sour odor, and result in deformation of the brain and spinal cord, rendering them unsuitable for histological and neuroanatomical study.

Compared to other techniques, the six-point injection technique is less commonly used due to its complexity and requirement for specialized equipment. However, this approach allows simultaneous arterial injection and venous drainage at six anatomical sites, potentially improving embalming fluid distribution. To simplify this technique, we developed a multi-branch injection tube for concurrent perfusion. This approach enables the injection of embalming fluid into 6 arteries simultaneously. This study introduces the six-point injection technique with venous drainage (MEM) as a safer, immersion-free alternative. Eliminating immersion may improve tissue quality and reduce chemical consumption for cadavers stored in our department. The objective was to compare MEM with the traditional method (PEM) in terms of joint mobility, tissue quality, and histological preservation.

## 2. Objectives

This study aimed to evaluate the effectiveness of a six-point injection embalming method with venous drainage (MEM) compared to the traditional two-point immersion method (PEM), focusing on joint mobility, tissue preservation quality, and histological integrity, while also assessing its potential to reduce chemical use and eliminate the need for immersion.

## 3. Materials and Methods

Ten cadavers, eight males and two females, were divided in two groups of 5 cadavers each. All cadavers were donated to the Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. The research was approved by the Research Ethics Committee, Faculty of Medicine, Chiang Mai University (Study code: ANA-2560-05071/ Research ID: 5071). The research was conducted in accordance with the regulations of the Faculty of Medicine, Chiang Mai University, Thailand. Informed consent was obtained from the donors' relatives. These cadavers had died from heart failure, pneumonia, or unknown causes. Cadavers who had died from diabetic mellitus and atherosclerosis were excluded. The bodies were stored at 0– 8°C and transported to the department within 48 hours.

### 3.1 Embalming Solution Formula

Cadavers in MEM and PEM groups were injected with the same embalming fluid. The formula consisted of solution 1 and solution 2 are given below:

Solution 1

| | | |
|---|---|---|
| Potassium nitrate (KNO3) | 1,000 | g. |
| Hot water | 6 | L. |

Mixed and left at room temperature until cold.

Solution 2

| | | |
|---|---|---|
| Glycerine | 4 | L. |
| 95 % Ethanol | 4 | L. |
| Phenol | 0.8 | L. |
| 40 % Formaldehyde | 3 | L. |

Solution 1 and 2 were mixed, this mixture was then injected into the cadavers.

**3.2 Embalming Methods for MEM**

The six regions targeted included the common carotid arteries (CCA) and internal jugular veins (IJV), the brachial arteries (BA) and veins (BV), and femoral arteries (FA) and veins (FV) (Mayer, 2011; Ajileye et al., 2018). The IJV, BV, and FV were incised, and the blood drainage tubes were inserted (Figure 1). The CCA, BA, and FA were incised, and the needles of the custom-designed injection tubes were inserted as shown in Figure 2. Approximately 30 % of the cadaver's body weight in embalming fluid was delivered using an air pressure machine set to 8 pounds per square inch, injecting fluid into the head, arms, and legs via either a modified six-point injection tube or a simple injection tube. While the embalming fluid was being delivered, blood was drained through the incised veins located parallel to the injected arteries. The four limbs, face, and abdomen appeared pale and swollen, indicating that embalming fluid had been thoroughly distributed throughout the body. After finishing this process, the valves of the simple injection tubes were closed. The cadavers were left on the table for one night. The next day, the needles from the injection tubes and blood drainage tubes were removed from the cadavers. Those incised arteries and veins were ligated. The incisions were sutured, and the entire bodies were cleaned with Dettol antiseptic solution. For the last step, cadavers were wrapped with plastic and placed in mortuary bags (Anderson, 2006; Mills, 2010). The cadavers were monitored monthly and stored at room temperature for one year to compare tissue and histological quality with those preserved using the PEM method.

**3.3 Embalming Methods for PEM**

The femoral arteries were incised, and the needles of the injection tubes were inserted into these vessels. One end of each needle was directed toward the head, and the other toward the feet. Approximately 30% of the cadaver's body weight in embalming fluid was delivered using an air pressure machine set to 8 pounds per square inch. After perfusion, all needles were removed from the cadavers. The vessels were ligated to prevent embalming liquid leakage. The incisions were sutured, and the entire bodies were cleaned with Dettol antiseptic solution. Lastly, the cadavers were immersed in embalming liquid pools for one year. The immersion solution contained formaldehyde and phenol at concentrations of 4% and 5%, respectively. The cadavers were checked once a month. The dissection study was conducted after one year of immersion.

**3.4 Measuring Range of Motion (ROM)**

The range of motion (ROM) of the shoulder joints, elbows, radioulnar joints, wrists, hip joints, knee joints, and ankles in various directions was measured using a standard goniometer. The angle between the immovable (anatomical) and movable planes of each joint was assessed. Angle measurements were performed both before the embalming fluid injection and after one year of preservation (Table 1).

**3.5 Histology Study**

Approximately 1 $cm^3$ of skin of the palmar surface of the right little finger, as well as samples of the pronator quadratus muscle, pancreas, and brain, were collected from all ten cadavers and processed for H&E staining. The quality of all tissue types from cadavers in the MEM and PEM groups were compared.

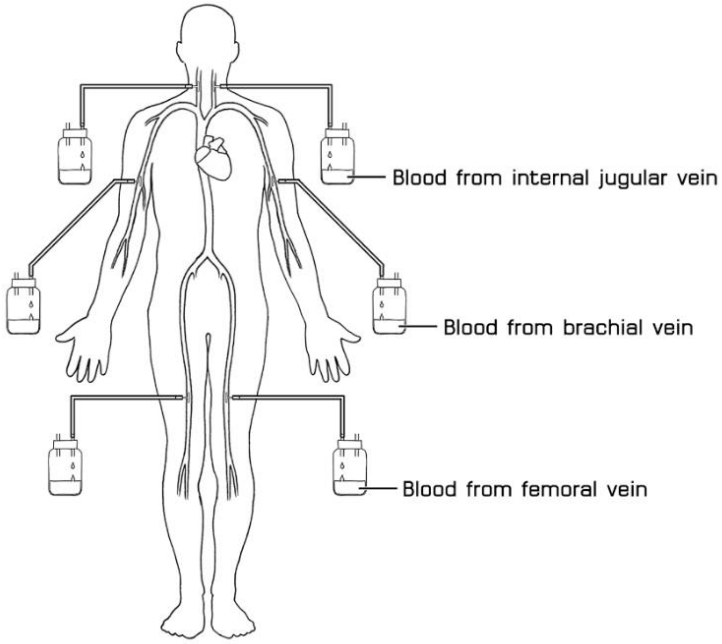


**Figure 1** The method of blood drainage from internal jugular, brachial, and femoral veins

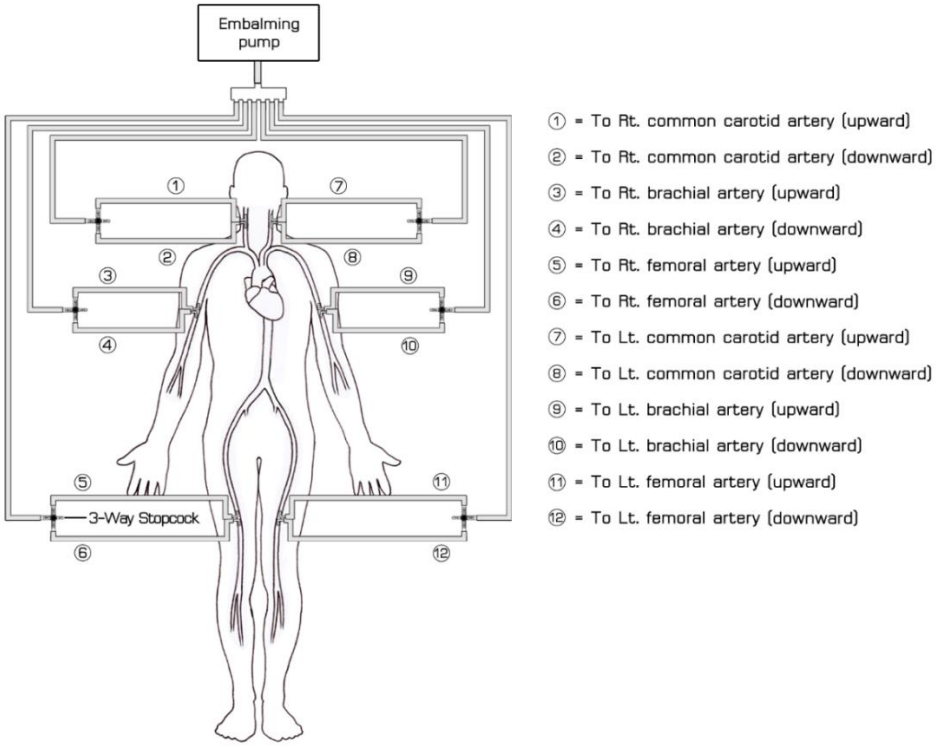


**Figure 2** Method for embalming fluid injection into common carotid, brachial, and femoral arteries

### 3.6 The Satisfaction Assessment of Dissection

All ten cadavers were dissected by ten individual dissectors. For each dissector, the skin on the palmar surface of all ten fingers and ten regions on the anterior thighs were randomly selected. The degree of softness and the color of skin, subcutaneous adipose tissue, and the muscles were scored ranging from 0 to10. The tissue quality of the thighs and fingers from fresh cadavers was used as a reference and scored equally (Figure 3A and 3B). Dry and hardened embalmed tissues from the thighs and fingers, which were unsuitable for classroom use, were given a score of 0 (Figure 3G and 3H). Scores were based on these indicators: for odor 0 = no odor; 1 = just perceptible; 2 = faint; 3 = easily noticeable; 4 = strong; 5 = very strong (Chen et al., 1999).

### 3.7 Data Analysis

The ROM between MEM and PEM groups was compared using an Independent Sample T-test. Scores for odor, color and softness of skin, subcutaneous adipose tissue, and muscles of cadavers embalmed using the modified and conventional methods were also analyzed.

## 4. Results

### 4.1 Comparison of the ROM of Joints

Before embalming, the ROM of the selected joints did not differ significantly (Table 1). After one-year of preservation, the ROM values of all selected joints were significantly reduced in both MEM and PEM groups, except for plantar flexion of the ankle joints in the MEM group. After one year of storage, the ROM of all selected joints in the MEM group was higher than in the PEM group, except for pronation of the radioulnar joints, adduction of the hip joints, and flexion of the ankle joints. The elbow and knee joints in both MEM and PEM groups were immobile (0°).

### 4.2 Comparison of Satisfaction in Dissection

The score on color of the subcutaneous adipose tissue and muscles were higher in the PEM group than in the MEM group, whereas the scores for skin color, skin softness, and muscle softness were higher in the MEM group. Cadavers in the MEM group were preferred for dissection over those in the PEM group (Table 2 and Figure 3). There was no significant difference in odor between the two groups, and the odor was generally mild.

**Table 1** ROM values in degree of joints in MEM and PEM groups

| ROM ($\bar{x}$) | | MEM | | | | PEM | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Before | After | Before | After | Before | After | Before | After |
| | | Rt. (n=5) | Lt. (n=5) | Rt. (n=5) | Lt. (n=5) | Rt. (n=5) | Lt. (n=5) | Rt. (n=5) | Lt. (n=5) |
| Shoulder joint | Flexion | 163 | 163 | 37.8 | 38.8 | 173 | 170 | 21.6 | 21.4 |
| | Abduction | 178 | 175 | 63.2 | 59.6 | 164.4 | 165 | 39 | 41.6 |
| | Internal rotation | 72 | 72 | 45 | 43.6 | 77 | 78 | 22.4 | 21.2 |
| | External rotation | 75 | 74 | 37.4 | 34.4 | 76 | 77 | 11 | 11.6 |
| Elbow joint | Flexion | 145 | 144 | 86.2 | 88 | 146 | 145 | 58.6 | 52.6 |
| | Extension | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Radio-ulnar joint | Pronation | 71 | 73 | 39.8 | 41.6 | 77 | 80 | 40.2 | 38.4 |
| | Extension | 77 | 78 | 44.6 | 43.6 | 79 | 80 | 22.2 | 24.4 |
| Wrist joint | Flexion | 71 | 72 | 33.6 | 33.8 | 78 | 72 | 24.6 | 23.6 |
| | Extension | 70 | 69 | 34.2 | 34.6 | 64 | 59 | 9.6 | 11.4 |
| Hip joint | Flexion | 124 | 126 | 25.8 | 24.8 | 123.6 | 129 | 14.8 | 14.4 |
| | Abduction | 46 | 44 | 20.2 | 22.2 | 48 | 48 | 16.4 | 17.4 |
| | Adduction | 24 | 24 | 7.6 | 9.2 | 24 | 27 | 52 | 82 |
| | Internal rotation | 34 | 33 | 13 | 12 | 35 | 35 | 7 | 8 |
| | External rotation | 45 | 45 | 39 | 40 | 40 | 43 | 11.2 | 16.2 |
| Knee joint | Flexion | 132 | 132 | 66.4 | 66.2 | 133.4 | 132 | 33 | 27.8 |
| | Extension | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ankle joint | Flexion | 37 | 36 | 28.6 | 30.4 | 45 | 46 | 34.2 | 34.4 |
| | Extension | 15 | 15 | -22.6 | -24.6 | 14.8 | 15 | -32.4 | -34.4 |

Note: Before, Before embalming; After, After one-year preservation; PEM, Present Embalming Method; MEM, Modified Embalming Method; n, number; $\bar{x}$, means

### 4.3 Comparison of the Histology of Thick Skin

Skin histology was compared between groups (Figures 4A and B). H&E staining of the finger skin from MEM cadavers clearly revealed all epidermal layers: stratum germinativum, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum (Figure 4A). In the dermis, acidophilic collagen fibers and basophilic fibroblast nuclei were clearly visible. Fibroblast nuclei were prominent. Blood vessels were well preserved. In the PEM group, the histological structure lacked distinct epidermal layers and a well-preserved dermis (Figure 4B).

### 4.4 Comparison of the Histology of Subcutaneous Adipose tissue

The MEM group showed better histological definition and preservation (Figures 4C and 4D). Fat cells in the MEM cadavers were intact (Figure 4C), and no red blood cells were observed in the lumens of blood vessels within the connective tissues. In the PEM group, fat cell membranes appeared irregular in contour (Figure 4D), and red blood cells were present in blood vessels throughout the connective tissues.

### 4.5 Comparison of the Histology of Skeletal Muscle

The pronator quadratus muscle of the MEM group exhibited polygonal muscle fibers with acidophilic sarcoplasm and basophilic oval nuclei. The endomysium was clearly seen. Most skeletal muscle cells in the MEM cadaver group were intact (Figure 4E). Mild shrinkage in some skeletal muscle fibers caused gaps between the cells and the endomysium. A small number of blood cells were found in the lumens of vessels located in the perimysium and endomysium. Most skeletal muscle fibers in the PEM group showed shrinking (Figure 4F). High numbers of blood cells were found inside the lumens of blood vessels located in the perimysium and endomysium. Thus, skeletal muscle in the MEM group was better preserved than in the PEM group (Figures 4E and F).

### 4.6 Comparison of the Histology of Pancreas

Pancreatic tissue from the MEM group displayed intact acinar cells. The cytoplasm was basophilic. Nuclei of centroacinar cells and intralobular ducts lined with simple cuboidal epithelium were clearly visible (Figure 4G). A small number of blood cells were found inside vesicle lumens. Most acinar cells in the PEM group were autolyzed, and their cytoplasm appeared mildly basophilic (Figure 4H). The epithelium of intralobular ducts was decayed and many blood cells were found within the lumens of blood vessels. Pancreatic tissue in the MEM group was better preserved than that in the PEM group.

### 4.7 Comparison of the Histology of Cerebral Cortex

The cerebral cortex of the MEM group revealed stellate shaped neurons. The circular nuclei and nucleolus were clearly seen. Cytoplasmic Nissl bodies were visible (Figure 4I). In addition to neurons, oligodendrocytes with small circular nuclei and clear cytoplasm were observed. Neurons and oligodendrocytes were located among neurites and glial cell processes, surrounded by lightly acidophilic fibers. There were no blood cells inside the capillary lumens in the cerebral cortex. The cerebral neurons of the PEM group were decayed and some neurons showed signs of shrinkage (Figure 4J). The cytoplasmic Nissl body was not seen. Blood cells in the capillary lumens appeared mildly acidophilic. As with previous tissues, the cerebral cortex in the MEM group was better preserved than in the PEM group.

**Table 2** The assessment of dissection between cadavers in MEM and PEM groups

| Evaluation | MEM (n=50) | | PEM (n=50) | | Significant differences MEMVSPEM (n=50) |
|---|---|---|---|---|---|
| | $\bar{x}$ | S.D. | $\bar{x}$ | S.D. | |
| Color of the skin | 7.4 | 12 | 6.4 | 1.6 | ** |
| Color of the subcutaneous Adipose tissue | 6.8 | 1.5 | 7.4 | 1.8 | * |
| Color of the muscle | 6.3 | 1.5 | 7.5 | 1.2 | ** |
| Softness of the skin | 7.4 | 1.4 | 6.6 | 1.6 | ** |
| Softness of the muscle | 7.5 | 0.9 | 6.9 | 1.3 | ** |
| Intensity of the odor | 2.1 | 1 | 2.2 | 0.8 | NS |

Note: MEM: Modified Embalming Method, PEM: Present Embalming Method, n: number, $\bar{x}$: means, S.D.: Standard Deviation, VS: Versus, NS: Not Significant ($P > 0.05$), *: Significant ($0.01 < P < 0.05$), **: Significant ($P < 0.01$)

## 5. Discussion

This study employed a modified embalming method ( MEM) that used multiple injections to address the problem of inadequate embalming fluid perfusion in cadavers (Mayer, 2011). We developed a multi- branch injection tube ( Figure 2) that can be inserted into six arteries and distributed the fluid in both the rostral and caudal directions. During the injection process, blood from all six veins running parallel to the arteries was drained. The present embalming method (PEM) used in the Department of Anatomy, Chiang Mai University, does not use blood drainage from the cadavers (Thaweekhotr et.al, 2022; Thaweekhotr et. al, 2023). Venous drainage creates space for arterial fluid, ensures even distribution, and helps prevent discoloration, odor, and gas formation. It also prevents tissue decomposition and reduces microbial activity ( Mayer, 2011). The MEM, which incorporates venous drainage, demonstrated superior preservation and morphology in skin color, subcutaneous adipose tissue, and muscle compared to the PEM. It also reduced the decomposition of tissue compared to those in the PEM.

The embalming fluid caused a reduction in ROM in cadavers from both groups. However, joints in the MEM group remained more mobile than those in the PEM group. The low flexibility of the selected joints of the PEM group may have resulted from the post-fixation in the embalming fluid pool for one year. In addition to formalin (Fox et al., 1985; Tolhurst, & Hart, 1990; Thavarajah et al. , 2012; Balta et al. , 2015b) , the embalming fluid pool also contains phenol. Phenol, an antibacterial and antifungal agent, also promotes tissue dehydration (Brenner, 2014).

A previous study reported that excess use of formalin and phenol caused oxidative damage, leading to tissue hardening and darkening ( Ajileye et al., 2018). The histology of skin at the finger edge of PEM cadaver group demonstrated a peeling of the epidermis. In addition, numerous decomposed blood cells were found in the dermis (Figure 5). Those blood cells obstructed embalming fluid flow, resulting in degraded skin histology.

All cadavers in the MEM groups were venous drained during the embalming injections, while those in the PEM received the embalming injections without venous drainage. Thus, venous drainage likely reduced blood and clot retention in most cadaveric tissues of the MEM group, allowing embalming fluid to circulate throughout the body, fix the tissues, and eventually enter the venous system. Histological appearances of tissues in the MEM group were better preserved and revealed no residual blood cells, unlike those in the PEM group. The brains of the MEM appeared firm and preserved their shape while most of the PEM were reddish, softened, and unfixed (Figure 6).

In addition to providing better tissue quality, the MEM method required less embalming fluid than PEM. MEM preserved cadavers at room temperature condition with no immersion. These protocols minimize chemical usage and may serve as a model for reducing hazardous material consumption.

Both the PEM and MEM groups exhibited mild odors, which remain a challenge in gross anatomy; future research may explore adjustments to the embalming fluid formulation.

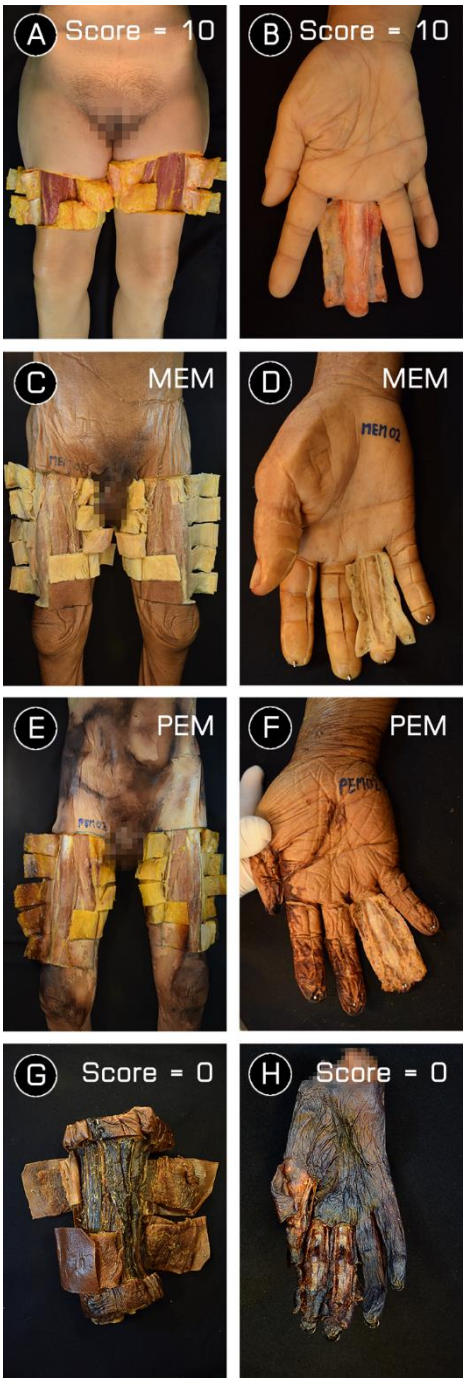


**Figure 3** Tissue quality scores for selected anatomical regions in cadavers preserved using the Modified Embalming Method (MEM) and Present Embalming Method (PEM). Scores ranged from 0 (dry, unusable specimens; panels G and H) to 10 (fresh cadaver-like quality; panels A and B). Representative regions from the anterior thigh and palmar surface of the fingers in MEM cadavers (panels C and D) and PEM cadavers (panels E and F) are shown.

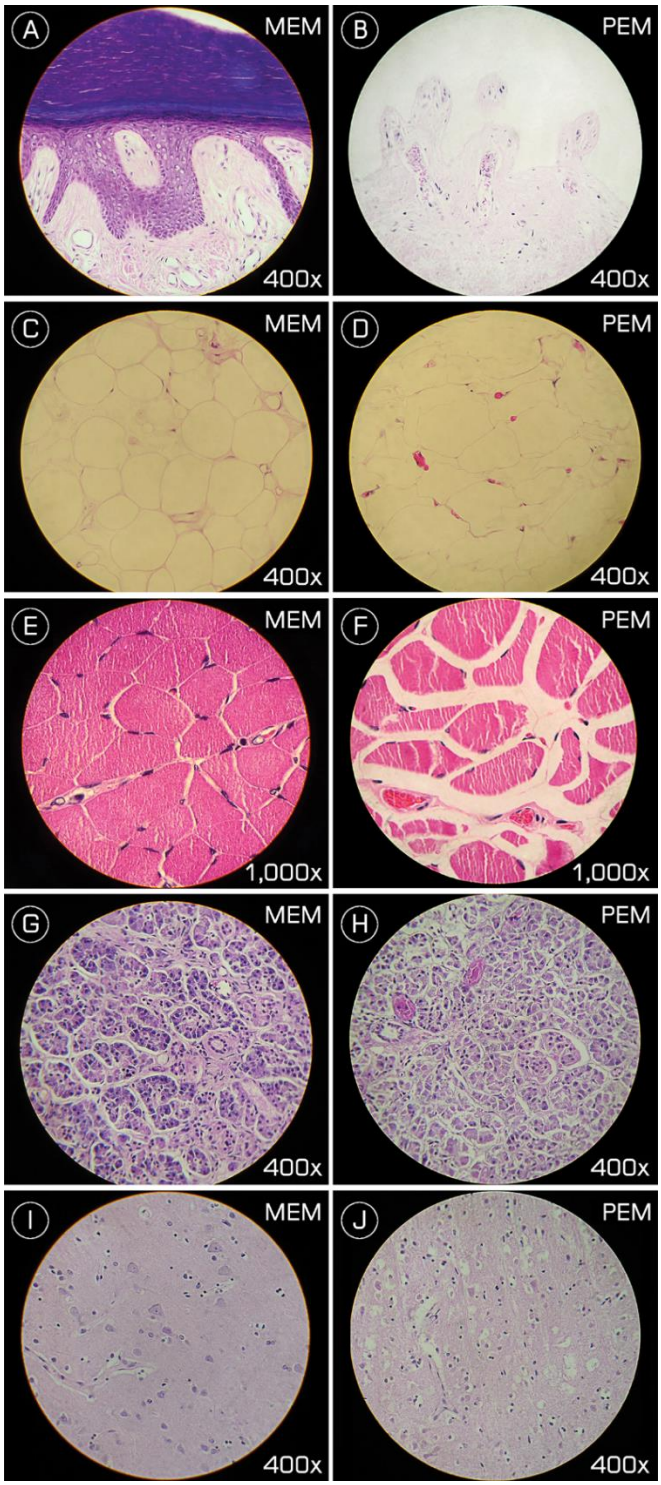


**Figure 4** Comparative histological analysis of tissues from cadavers preserved using the Modified Embalming Method (MEM, left column) and the Present Embalming Method (PEM, right column). Shown are representative sections of the skin (A, B), subcutaneous adipose tissue (C, D), skeletal muscle (E, F), pancreas (G, H), and cerebral cortex (I, J), demonstrating superior preservation and structural integrity in the MEM group.

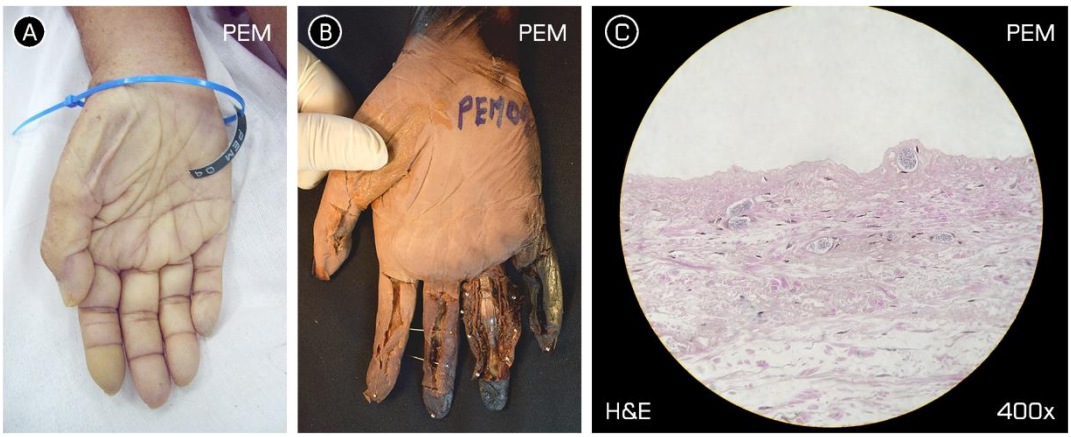


**Figure 5** Condition of the right hand of a cadaver from the PEM group at different time points. (A) Appearance at 15 hours post-embalming fluid injection. (B) Appearance after one year of immersion in the embalming fluid pool. (C) Histological section of the finger skin edge after one year of immersion, showing structural degradation

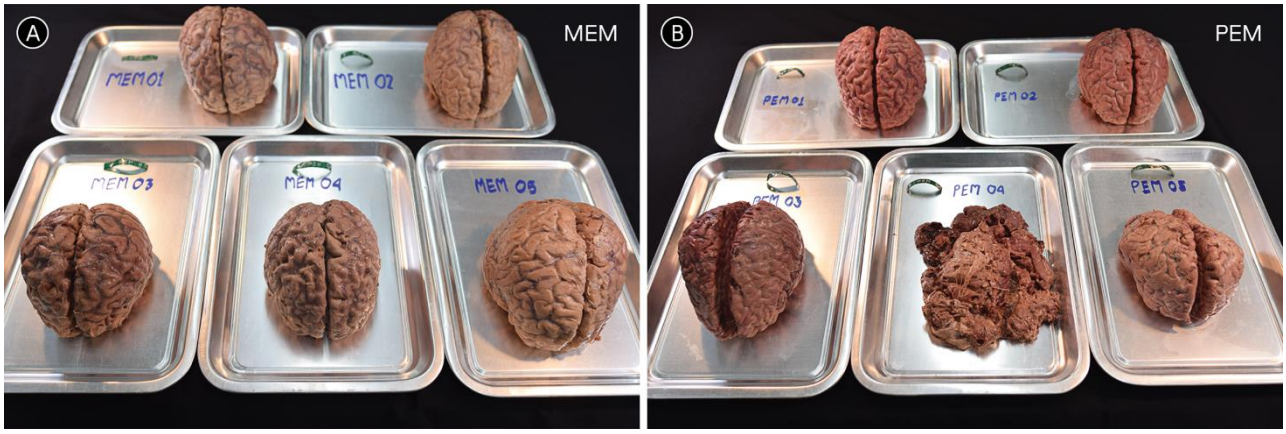


**Figure 6** Gross morphology of brains from cadavers preserved using the Modified Embalming Method (MEM, A) and the Present Embalming Method (PEM, B)

## 6. Conclusion

Embalming fluid injection using a six-point technique combined with venous drainage ensured uniform fluid distribution throughout the body. This method, referred to as MEM, produced well-preserved cadavers suitable for gross anatomy, neuroanatomy, and histological studies. Instead of being immersed in an embalming fluid pool, the injected cadavers were stored in plastic bags at room temperature. This approach reduced both the cost and use of hazardous chemicals.

## 7. Acknowledgements

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## 8. Conflict of Interest

The authors declare no conflict of interest.

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