

MODIFIED LOW-DOSE FORMALIN PRESERVATION: IN THE ANATOMICAL-SURGICAL DISSECTION & PROSECTED ANATOMICAL MODELS: A CASE REPORT

Fifi Veronica^{1*}, Resti Gradia¹, Lulu L. Fitri²

¹Department of Basic Sciences, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia

²School of Life Science and Technology (SITH) ITB, Bandung, Indonesia

ARTICLE INFO

Corresponding author :

Fifi Veronica
Universitas Padjadjaran
Email:
fifi@unpad.ac.id

Kata kunci:

Preservasi Modifikasi Dosis
Rendah Formalin
Diseksi anatomi klinis

Keywords:

Modified Low Dose
Formalin Preservation
Anatomical and clinical
dissection

Original submission:

Oct 18, 2024

Accepted:

Dec 3, 2024

Published:

January 20, 2025

ABSTRAK

Perkembangan preservasi kadaver telah mengalami perkembangan yang pesat. Banyak penelitian yang menemukan efek samping jangka panjang pada penggunaan bahan pengawet pada masa lampau. Perlambatan pembusukan, mencegah kekerasan jaringan, mempertahankan warna jaringan semirip dengan aslinya, menjaga kelenturan jaringan serta menghambat perkembangan bakteri dan jamur pada jaringan yang dipreservasi adalah tujuan utama dari suatu pengawetan. Formula baru dalam preservasi sudah ditemukan dengan menggunakan formalin dosis rendah dan mengkombinasikannya dengan beberapa bahan pengawet lainnya. Formula ini sudah digunakan pada beberapa aktivitas diseksi anatomi-klinis dengan menggunakan kadaver atau pada pembuatan model diseksi dengan pemeliharaan yang mudah dan murah.

ABSTRACT

Modified Low Dose Formalin Preservation: In the Anatomical-Surgical Dissection & Prosected Anatomical Models: A Case Report. The development of cadaver preservation has been fast-paced. The subtraction of formalin dose in embalming has been used since many negative factors were found in long-term usage. Preventing putrefaction progress on the cadavers, ensuring no risk of infection when in contact with a dead body, preventing hardening and retention of color tissue and organs, preventing desiccation, inhibit fungal or bacterial growth are the main goals of embalming for anatomical purposes. The new formula has been developed by using low-dose formalin, ethanol, phenol, and sodium chloride (Modified Low-Dose Formalin). This method can be used in practical anatomical-surgical dissection using either cadavers or anatomical models with low-cost maintenance.

INTRODUCTION

The development in the School of Medicine will always be connected to 3 important matters: curriculum development, human resources, and laboratory facilities. All of which are in coherence with each other. Curriculum development is related to the number and qualification of lecturers and the school's laboratory facilities. One of the most important things in the development of a laboratory facility is cadaver preservation.^{1,2}

In medicine, the development of cadaver preservation has been a fast-paced advancement. Modifications have been made to the type and amount of compounds like formalin, ethanol, glycerin, chloral hydrate, calcium and sodium chloride, and phenols used in various cadaver preservation research.³ The subtraction of formalin dose in embalming has been used since many

negative factors were found in long-term usage.⁴ This method replaces high formalin concentration with a low dose of formalin to preserve tissue.^{5,6}

Preventing putrefaction progress on the cadavers, ensuring no risk of infection when in contact with a dead body, preventing hardening and retention of color tissue and organs, preventing desiccation, inhibit fungal or bacterial growth are the main goals of embalming for anatomical purposes.⁷ Division of Anatomy in the Faculty of Medicine of Universitas Padjadjaran has been developing a method of cadaver preservation with a combined formula of low-dose formalin, ethanol, phenol, and sodium chloride for 5 years. Research with a preservation method using a modified low dose of formalin (MLF) in experimental animals has been conducted to test the formula's effectiveness. In Woanshian's research, different colors were found in gross anatomical samples of muscle and intestine between preservation using conventional formalin (CF) and MLF. The method using CF was a total of 37% formalin. The sample color with MLF was seen to be closer to its natural color than in the sample using CF. Nurmalasari's research also observed histological pictures and microanatomical shrinking from the intestine, skin, and muscles by comparing the 10% formalin solution and Larsen's solution. Larsen's solution has similarities with MLF but is slightly different in the composition of the compound. The study using Larsen's method achieved 10% better results in microscopic, macroscopic, and microbiology images than formalin.⁸⁻¹⁰

Division of Anatomy in the Faculty of Medicine of Universitas Padjadjaran is researching and testing the MLF method in cadaver samples, analyzing microanatomical dissection, biomechanics, histology, and microbiology. Several important matters that the Faculty have been doing are developing dissection training, designing anatomical models, and developing anatomical curricula using MLF in preservation. The division of Anatomy in the Faculty of Medicine of Universitas Padjadjaran has recently started a program to train general practitioners and specialists. Numerous interdisciplinary training courses were established in collaboration with the clinical department at Hasan Sadikin Hospital or the anatomical association.

The MLF formula positively impacts anatomical development in the Faculty of Medicine of Universitas Padjadjaran. Implications of the MLF method can be used in practical anatomical courses using either cadavers or anatomical models. To come as close as possible to clinical reality, we introduced new embalming techniques that allow simulation establishment or even new surgical methods. This study aims to evaluate the effectiveness of The MLF for cadaver preservation and its implication for medical education and research.

Case Report:

Modified Low Dose-Formaline (MLF) Preservation

A cadaver is the main educational tool that is intended for the dissection and demonstration of protected specimens with strong impressions. MLS offers improved tissue quality compared to conventional formalin methods.

Cadavers fixed by conventional procedures by using mainly formalin for conservation are only of limited use for practical surgical courses due to profound changes in color, strength, and fragility of organs and tissues.

In MLF preservation, 37% of formalin is still used for fixation but in a minimal concentration. A previous study used saturated salt solutions to embalm veterinary gross anatomy models. But in cases using embalming in cadavers for dissection and anatomical models that have been used for several years, the saturated salt solution needs a combined mixture with other compounds.¹¹

Division of Anatomy in the Faculty of Medicine of Universitas Padjajaran has a new protocol for embalming cadavers, using a low dose of formalin 37% and a saturated salt solution, as well as for fixation. Phenol crystal is disinfected, and ethanol is used to preserve tissue plasticity. The cadavers are perfused by means of a femoral or carotid artery with a solution containing 20 liters of stem solution—additional solution injection at the tips of the fingers, 10 ml every finger. The cadavers are stored for approximately 12 weeks in plastic bags and kept in a refrigerator at -9°C and closed with a zipper. The cadavers may be used for months to 2 years. This method has the advantages of anatomical practice and low-cost maintenance.

Modified Low Dose-Formaline (MLF) Preservation: Anatomical-Surgical Training Courses Dissection

The formalin content of the fixation solution is drastically reduced, with a final concentration of 7.4%. The low-odor embalming technique produces well-preserved organs and tissues concerning color, consistency, and transparency. The procedure affords high standards of preservation with good disinfecting efficacy and without releasing harmful substances into the environment. The excellent quality of preservation is visualized in Fig 1.



Fig.1 Anatomical lower limb dissection and Blunt fiber dissection. This figure shows anatomical dissection layer by layer. Superficial and deep fascia can be exposed until it reaches the sciatic nerve. Tissue consistency and color are natural. Cerebral cortex-sparing fiber dissection enabled us to isolate white matter from grey matter. The fibers run at the parietal and frontal operculum.

Blunt fiber dissection or tractography is classical fiber dissection of post-mortem human brains. This dissection enables us to isolate a fiber tract by removing the cortex and overlying white matter. Fig. 2 is a blunt fiber dissection using MLF after at least 15 days of perfusion and freezing at -9°C . The fiber can be seen under the microscope and visualized. Several different clinicians can use the specimen from MLF. It is an important tool for neurosurgical and neuroanatomy research.¹²



Fig.2 Visualized prosected anatomy models.

Prosected anatomy models (superior extremity). Fig.3a shows superficial fascial and muscle. Fig. 3b&c show the musculocutaneous nerve below the brachialis muscle.

Projected anatomical models with MLF preservation have several advantages. Besides being odorless, the tissue is like in its natural color. The student can experience a high impression of the model. The colors of muscles, fasciae, fatty tissue, nerves, and vessels were evaluated, and the life-like tissues of MLF cadavers were impressive.¹³ There were no obvious or disturbing smells or signs of putrefaction of the MLF cadavers. After dissection, the models were sunk in a 10% formalin solution and will be rinsed 2 hours before use. MLF is a sustainable and relatively affordable soft cadaver embalming method. Its application is the same as conventional methods and does not need new equipment.

Using formalin solution in embalming has advantages such as being degradable, inexpensive, fully potent of germicide, and fast action, as we can see in embalming tissue. Besides that, formalin also has disadvantages: unpleasant and irritating odor, tissue hardening and discolorations, and its carcinogen effect.^{14,15}

Using low doses of formalin, saturated salt solution, phenol crystal, and ethanol for preservation in embalming is a new formula with many advantages in medical education. Formalin has antiseptic properties and bactericidal, fungicidal, and insecticidal properties to prevent the entry of decay organisms. Although formalin is the best tissue fixative, its use is commonly associated with extreme rigidity, fixes discolorations, dehydrates tissues, and has an unpleasant odor. Exposure to high-level formalin over a long time in the dissecting laboratory can cause irritants and cause mild eye and mucous membrane complaints.¹⁶ Shifting from a huge formalin solution with low doses of formalin – ethanol had several changes like odor, the color and consistency of the tissue, and the number of bacterial growths in muscle and colon rat preservative tissues. Formaldehyde in formalin's preservative formula makes the grayish color because the formalin converts hemoglobin into methemoglobin and is also involved in the oxidation process. A combination of low doses of formalin with ethanol has enough retention of the tissue color.¹⁷ In previous studies, ethanol was used as a solvent of alcohol and as an anti-infective potent agent. Combining ethanol with glycerine could denature proteins reversibly, cause the coating to hydrate the tissue's structure, and disrupt the structures.¹⁸

Phenol or carbolic acid is a part of the component in the Modified Low Dose Formalin formula. Phenol has disinfectant properties, which are bacteriostatic in small concentrations. Phenol can potentially affect deactivate enzymes within the cell and cell permeability. In a previous study, phenol was used in embalming as a medium to penetrate the formula because of its superior penetration ability.^{10,12}

An additional component in MLF is salts. Previous studies stated that salts had strongly stabilized proteins.¹⁰ Humectants and wetting agents such as glycerine are used as part of MLF. Previous studies showed that a combination of a small amount of formalin and glycerine has a more powerful disinfectant effect compared to formaline alone.^{19,20} Comparison between MLF and other preservation methods has its own risks and benefits, but using MLF for long-term duration has advantages in better preservation.

Although the previous studies were only in rat tissues, the results showed that MLF has a potential antibacterial effect, the same as the formalin formula. MLF inhibits the growth of bacteria because it contains 70% ethanol as an antiseptic. A reduction amount applied formalin below 1,5% increases the risk for infection. Testing for antimicrobial, biomechanical, histological, and usability in MLF formula on human embalmed tissue must be done. However, several tests have been done on animal sample models.

Conclusion

Modified Low-Dose Formalin can be used as an alternative to conventional fixed formalin.

ACKNOWLEDGEMENTS

This research were funded by Universitas Padjadjaran Internal Grant 2020

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest with the contents of this article.

REFERENCES

1. Roxburgh M, Darrell J.R. Evans, Evans DJR. Assessing Anatomy Education: A Perspective from Design. *Perspective Article*. 2021;20–1.
2. Khalil MK, GEL, L V., Giannaris EL, Lee V. Integration of clinical anatomical sciences in medical education: Design, development and implementation strategies. *J Clinical Anatomy*. 2021 May 3;34(5):785–93.
3. GEAVLETE B, POPESCU R, IORDACHE V, GEORGESCU D, GEAVLETE P. Innovative Cadaver Preservation Techniques: a Systematic Review. *Maedica - A Journal of Clinical Medicine*. 2023 Mar 15;18(1).
4. Bhanarkar AK, Ray UP. Embalming with Formalin-Benefits and Pitfalls. *Scholars International Journal of Anatomy and Physiology* Abbreviated Key Title: *Sch Int J Anat Physiol*. 2022;5(3).
5. Lombardero M, Yllera MM, Costa-e-Silva A, Oliveira MJ, Ferreira PG. Saturated salt solution: a further step to a formaldehyde-free embalming method for veterinary gross anatomy. *J Anat [Internet]*. 2017 Aug 1 [cited 2024 Dec 24];231(2):309–17. Available from: <https://pubmed.ncbi.nlm.nih.gov/28542788/>
6. Nam SM, Moon JS, Yoon HY, Chang BJ, Nahm SS. Comparative evaluation of canine cadaver embalming methods for veterinary anatomy education. *Anat Sci Int [Internet]*. 2020 Sep 1 [cited 2024 Dec 24];95(4):498–507. Available from: <https://link.springer.com/article/10.1007/s12565-020-00547-x>
7. Arce JE, Contreras E, Vaghani P, Goyal K, Balta JY. The Dissection Experience of Orthopedic Resident: A Comparison between Soft-Preservation and Formalin-Fixation Embalming Techniques. *The FASEB Journal [Internet]*. 2022 May [cited 2024 Dec 24];36(S1). Available from: <https://onlinelibrary.wiley.com/doi/full/10.1096/fasebj.2022.36.S1.R5264>
8. Shian SW, Soenggono A, Pramod SV. Comparison of Tissue Preservation using Formalin and Ethanol as Preservative Formula. *Althea Medical Journal [Internet]*. 2016 Sep 30 [cited 2024 Dec 24];3(3):359–63. Available from: <https://journal.fk.unpad.ac.id/index.php/amj/article/view/880>
9. Mrožek A, Sędzik M, Rubin J, Buras S, Tarkowski V, Domagała Z. Cadaver preserving methods – is it possible to do anything better? *Medical Journal of Cell Biology*. 2022 Dec 1;10(4):124–8.
10. Pustaka Ilmiah Universitas Padjadjaran » Blog Archive » PERBANDINGAN FORMALIN 10% DAN MODIFIKASI LARUTAN LARSEN DALAM PENGAWETAN JARINGAN TIKUS GALUR WISTAR YANG DITINJAU DALAM STRUKTUR MAKROSKOPIK DAN JUMLAH KOLONI BAKTERI [Internet]. [cited 2024 Dec 24]. Available from: <https://pustaka.unpad.ac.id/archives/126877>
11. Gosomji IJ, Omirinde JO, Hena SA, Wanmi N, Azeez IA. Saturated Salt Solution an Alternative Reagent in Reducing Formaldehyde Concentration in Embalming. 2018 Jan 5 [cited 2024 Dec 24]; Available from: <http://irepos.unijos.edu.ng/jspui/handle/123456789/2391>

12. Shah A, Jhavar S, Goel A, Goel A. Corpus Callosum and Its Connections: A Fiber Dissection Study. *World Neurosurg* [Internet]. 2021 Jul 1 [cited 2024 Dec 24];151:e1024–35. Available from: <https://pubmed.ncbi.nlm.nih.gov/34033953/>
13. Soonklang N, Saowakon N. Evaluation of formaldehyde exposure among gross dissection after modified embalming solution and health assessment. *Environmental Science and Pollution Research* [Internet]. 2022 Sep 1 [cited 2024 Dec 24];29(43):65642–54. Available from: <https://link.springer.com/article/10.1007/s11356-022-19704-y>
14. Tiruneh C. Acute adverse effects of formaldehyde treated cadaver on new innovative medical students and anatomy staff members in the dissection hall at Wollo University, Northeast Ethiopia. *Adv Med Educ Pract* [Internet]. 2021 [cited 2024 Dec 24];12:41–7. Available from: <https://www.tandfonline.com/action/journalInformation?journalCode=dame20>
15. Lang I, Bruckner T, Triebig G. Formaldehyde and chemosensory irritation in humans: a controlled human exposure study. *Regul Toxicol Pharmacol* [Internet]. 2008 Feb [cited 2024 Dec 24];50(1):23–36. Available from: <https://pubmed.ncbi.nlm.nih.gov/17942205/>
16. Kang DS, Kim HS, Jung JH, Lee CM, Ahn YS, Seo YR. Formaldehyde exposure and leukemia risk: a comprehensive review and network-based toxicogenomic approach. *Genes Environ* [Internet]. 2021 Dec 1 [cited 2024 Dec 24];43(1). Available from: <https://pubmed.ncbi.nlm.nih.gov/33845901/>
17. Sajjacharoenpong K, Chuesiri P. Effectiveness of embalming solutions with partial replacement of formaldehyde and phenol with ethanol and sodium chloride on mice over 18 months. *The Thai Journal of Veterinary Medicine* [Internet]. 2020 Jun 1 [cited 2024 Dec 24];50(2):233–8. Available from: <https://digital.car.chula.ac.th/tjvm/vol50/iss2/14>
18. Oruç Zİ, Turan E. Investigation of the use of gelatine-glycerine and gelatine-polyvinyl alcohol mixtures as tissue consistency preservatives in cadavers fixed with modified SEFS solution. *Ann Anat* [Internet]. 2024 Jun 1 [cited 2024 Dec 24];254. Available from: <https://pubmed.ncbi.nlm.nih.gov/38408528/>
19. Sam F, Francis DV, Michael JS, Ganesan MP, Rabi S. Replacing formalin with phenoxetol as an immersion medium in a human cadaveric storage facility – A feasibility study. *Current Medical Issues*. 2024 Jan;22(1):7–13.
20. Watanabe M, Yoneyama Y, Hamada H, Kohno M, Hasegawa O, Takahashi H, et al. The Usefulness of Saturated Salt Solution Embalming Method for Oral Surgical Skills Training: A New Cadaveric Training Model for Bone Harvesting. *Anat Sci Educ* [Internet]. 2020 Sep 1 [cited 2024 Dec 24];13(5):628–35. Available from: <https://pubmed.ncbi.nlm.nih.gov/31608585/>