ABSTRACT

Art of embalming as practiced by Egyptians about 3000 years ago transformed into embalming science of modern ages with the use of formaldehyde as a preservative solution. Subsequently, the search for an ideal embalming preservative solution continues to date because of the health hazards associated with formaldehyde preservation of cadavers. Alternative preservative methods and solutions suitable for making different skill training models with the specific requirements of pliability have also experimented. The literature has documented various solutions like Thiel’s solution, cryopreservation, N-Vinyl-2-pyrrolidone, Ethanol–glycerin and Fix 4life solution as alternatives to formaldehyde preservation. This review is an attempt to have an overview comparison of all the recent alternate embalming methods applicable for developing skill training cadaveric models with an aim of reducing formaldehyde usage in preservation.

Key Words: Embalming; Cadaver; Preservation; Thiel embalming; Phenoxethanol; Salt solution

INTRODUCTION

The physical immortality is a never-ending quest for human beings. Over the history of mankind, different methods of preserving the body before and after death were being practiced. All those preservations of dead body were in the realm of religion and belief of afterlife, as practised since 3000 years for Egyptian mummies [1]. The process of near-normal preservation of human cadaver with chemical resulting in minimal decomposition and microbial growth is called embalming [2]. This embalming is of two types namely funeral and medical embalming. In either of these types, the body tissues were fixed by chemical preservative fluid. The long duration of preservation is the aim of medical embalming whereas in funeral it’s for a shorter temporary duration. The ideal preservation fluid has been considered to exhibit antibacterial, fungicidal, non-toxic and non-discolouring properties, still retaining the flexibility of joints and tissues. Metaphorisation of “art of embalming” to “science of preservation” had resulted in experimentation of different compositions, mixtures and preservative solutions for preservation over the recent years.

Changing trends in medical education after Flexner’s report had necessitated formal skill training of procedures a reality. The congruent shift in patient safety measures, ethical and legal reviews in patient care and advancement of surgical procedures paved way for development of skill training models for medical graduates [3–6]. Apart from the specific requirements in each of these skill training models, achieving “near life” flexibility and pliability in cadavers happened to the common requirement [3]. This review was an attempt to provide a comprehensive enumeration of preservative solutions ideal for skill training models, from the multitude of available embalming fluids.

Formaldehyde

Apart from different natural preservatives used in ancient times, formaldehyde has been in vogue as the primary preservative in embalming in the modern era [7]. Altered DNA methylation, histone modification & changes in microRNA expression are the epigenetic effects and reduced counts of NK cells, regulatory T cells & CD8 effector memory T cells were also noted due to inhalation of formaldehyde [8]. Repeated reports of cytotoxic, carcinogenic and mutagenic effects in embalming personnel and also darkening of preserved cadavers with formaldehyde [8]. Repeated reports of cytotoxic, carcinogenic and mutagenic effects in embalming personnel and also darkening of preserved cadavers with formaldehyde [8].

It was observed that phenoxyethanol lacks the efficiency to be an early preservative solution for formalin preservation in human cadavers.

Formaldehyde as an alternate preservative solution for formalin preservation in human cadavers since 2010 [13].5

Glutaraldehyde

Harries and Tank synthesized glutaraldehyde in 1908. It was first used as a fixative for embalming in 1955. It has disinfective and sterilizing properties effective against the majority of the microorganisms including viruses and spores. Also, the preserved cadavers exhibited required pliability. But the slow perfusion of Glutaraldehyde into the cadaveric tissue necessitating prolonged time for fixation and preservation limited its usage. Moreover, Glutaraldehyde vapors used for longterm were observed to cause respiratory irritation and localized edema for the exposed personnel. These disadvantages led on to decision of European Commission, Brussels to enforce discontinuation of glutaraldehyde preservation for cadavers since 2010 [13].

Phenoxethanol (phenoxyl, phenoxylethyl alcohol) embalming

The Phenoxethanol, an aromatic oily liquid with the appreciable antiseptic property was used mainly for zoological specimen fixation apart from its normal usage in antiseptic, bactericidal creams, cosmetics and insect repellents [14]. Trials were done to find the effectiveness of this compound as an alternate preservative solution for formalin preservation in human cadavers.

It was observed that phenoxethanol lacks the efficiency to be an early fixative of tissues because of its limitation in preventing autolysis of cells.
This warranted usage of additional early fixative like formalin along with phenoxethanol. In this method of preservation formalin fixation and phenoxethanol preservation in two staged process was done. During dissection also the cadaver and other dissected parts were subjected to treatment and covering with sheets soaked in the same compound. Direct non-exposure of skin personnel was important for preserving the cadaver without specific precaution advised during handling of cadavers embalmed by this method [15]. Subsequent studies suggested the need for substitution of formalin in cadaver fixation and increasing usage of phenoxethanol for cadavers, museum specimen and even histological fixation of tissues [15]. Non-pungent odour and almost negligible local as well as systemic toxicity by phenoxethanol were considered to be its advantages [16]. The 600 litres of chemical usage for every single cadaver, almost 10 months of fixation process, need for continuous immersion of specimens to prevent mould formation and sporadic reporting of instances of immunosuppression of exposed health personnel were claimed as limitations of using this preservation for skill training models [17].

Fresh frozen (cryopreservation) embalming

The Low temperature blocks the biochemical mechanisms that trigger cell death cascade in cells. This basic scientific principle was put into use in the fresh frozen method of embalming cadavers with a subzero temperature of 77-kelvin degrees. This method also used vitrification solutions that could crystallize the water in the cadavers [18]. This method was found to be exemplary in human Oocyte preservation with a triple sucrose solution as an adjuvant [19].

Laessen solution(modified)

The original formula of Larssen solution and is modified known as ‘Modifited Larssen solution’ (MLS). In MLS, along with other salts, glycerol was added and formalin was reduced in comparison to original solution. When compared to formalin-fixed cadavers it was found that the flexibility and working comfort ofMLS fixed cadavers was satisfactory enough to utilize for surgical training courses. The need to thaw the cadaver at room temperature for at least 3 days before actual utilization of the long term preserved cadavers was considered as a negligible limitation for MLS [20].

Thiel embalming

By 1992, a method of soft embalming using two types of solution one for intravenous and another for immersion was put forward by Professor Walter Thiel. In this technique, the organ was Perfused through specific tubes and cavities were intravenously injected [21]. Extensive successful usage of this method over the years had perfected this technique [22-26]. Certain documentation of modification to the Thiel’s solution concerning the concentration of chemicals and mixture composition also exist [27-31].

Quest for effective method of musculoskeletal preservation tested the suitability of Thiel’s technique. The distinctive tissue plane demarcation, realistic joint architecture with the flexibility of joints proved to be beneficial outcomes observed in this method [32]. Another study detailed the chemical action of Thiel’s solution upon musculoskeletal elements of the cadaveric specimen employing an animal model. The study demonstrated that the boric acid used in Thiel’s solution acted as a denaturing agent to myofibrils resulting in fragmentation and degradation of muscle fibres. Yet, tendon was noted to be non-disrupted by this method [33].

The property of Thiel’s method in restoring hydration of cadaver was the distinctive factor that made “soft embalming” to be considered for developing different skill training models [24]. It was also found to be appropriate for human cadaver used in making a flap and microvascular suture training specimens [22]. Simulation model training with fibreoptic tracheal intubation found cadavers preserved using Thiel’s solution proved to be superior to even manikins [34].

Various studies had observed that cadavers embalmed with Thiel’s method were reasonably accommodative for diverse sophisticated surgery training models when compared to routine formalin-fixed cadavers [35-39]. Hands-on training models for various laparoscopic surgery, image guided training models developed using Thiel’s cadaver with documented effectiveness[40].

In midst of all these astounding effectiveness in skill training model development, Thiel’s fixation was claimed to have high electrical conductivity as a considerable limitation. Presence of potassium nitrate, ammonium nitrate and sodium sulphite in Thiel’s composition was ascribed as the reason for this phenomenon. An attempt was done in altering the chemical composition and concentration without compromising the advantages provided by Thiel’s solution. In this alternate method, specimens were subjected to the two-staged process where in they were submerged in original Thiel’s solution for 28 days only and followed by the 2 weeks usage of the modified solution [41]. Even though, this modification eliminated the electrical conducting phenomenon of Thiel’s fixation, the difficulty in internal visceral dissection stayed to be challenging in specimens preserved by this method. Thus this method of cadaver preservation was found to have limitation for use among students for routine dissection in anatomy laboratory [29].

Saturated salt solution (ss) embalming method

In the pursuit for yet another effective alternative to formaldehyde fixation of cadavers, SSS with its different salts was experimented [30]. This solution was used in cadavers used for orthopedic surgical training, anatomy dissection and advanced trauma surgical training workshop model [42-43]. An advanced “cadaver based educational seminar for trauma surgery” found SSS method of fixation to be advantageous with reduced level of formaldehyde exposure [44]. Evaluation of comparative fixation efficacy in cadavers among the three common solutions, SSS, Thiel solution and formalin solution for skills training concluded that SSS exhibited a greater level of maintenance of tissue integrity. It also found that SSS cadavers were of low infectious risk and reduced formalin-induced consequences to the persons handling cadavers. And so, cadavers embalmed by SSS were declared to be proper for developing trauma surgical skills training models [45].

Ethanol-glycerin fixation method

In this method, the post embalming preservation was done at 5 degrees with cadavers wrapped with polyethylene foil. This method reported preservation of body without any evidence of putrefaction occurring in the embalmed cadaver up to 3 years duration [46]. Increased flexibility of cadaveric joints aiding easy approach to challenging regions like axilla and perineum was proposed as the prime advantage of this method. The distinction between nervous and vascular structures and detailed internal organs architecture were also observed to be preserved in this ethanol -glycerin method of fixation. Usage of this fixation method for finer preservation of cadaver without added health determents was reported by few studies [47]. Superiority of ethanol-glycerin fixation method for routine student dissection in contrast to Thiel’s embalming technique was also documented. Nevertheless, the same study reported the limitation of the ethanol-glycerin fixation for the preservation of cadaveric workshop specimens [48].

Shellac mixture

Shellac is an animal origin natural polymer, derived from secretions of Lac insects named Laccifer Laccra. The resin was secreted forms the covering of the insect larvae. The lac was obtained from the host trees by cutting branches containing resinous insects and grinding with processing. Shellac is soluble in alcohol and alkaline solutions but insoluble in water. It has very low water and acid permeability. Commonly this polymer was used in food industry, ceiling, glossing and also in pharmaceutical industries. The use of shellac mixture for embalming of cadavers was observed to be safe and environment friendly. But slight brown discoloration of skin and not tested for skill training model development were its limitations [49].

Goyri-o-neillembalming solution

The contents of this solution were toxic by nature. As they did not produce any toxic vapors at room temperature and were not harmful on touch unless directly it was deemed to be fit for usage. No change in color of skin with minimal change in the elasticity and minimal increase in the swelling of tissues were reported in cadavers embalmed using this solution. Retention of flexibility of joints as similar to live state with maintenance of structural neurovascular integrity and high morphological correlation with the living tissues even on scanning electron microscopic examination were significant advantages of using this solution for preparing skill training models [50].

Natekar and Desouza Solution

The contents Long duration of preservation of undissected cadavers, free of fungus and maggots upto 5years was considered to be the prime factor for using this preservation method. It was also observed that skin exhibited a natural look. The fascia, arteries and muscles appeared red in color which made differentiating them easy in skill training specimen [51]. The cost effectiveness and environmentally friendly nature of the solutions composition added to its advantage.

Nviny1-2-pyrollidene (nvp) embalming

The cadaver fixation for approaching normal basalts by endoscopic transanal
route was tested by utilizing a "preserve" solution [52]. The cadavers were observed to have soft, pliable body cavities, transparent connective tissue with reduced subcutaneous fat, clearly delineated vessels, nerves and flexible joints. This study finalized that NFP fixation of cadavers could be considered beneficial to routine formalin fixation when the aim of fixation was for skills training and development of innovative models involving cavities [53].

Fix 4 life solution

The quest for ideal cadaver preservation for airway management simulation training concluded that fix for life (FIL) solution made up of a non-hazardous mixture of aldehydes and manikin cadavers were the most suitable method [54]. This review of merits and demerits of different cadaver preservation methods in usage for skill training model development impressed upon the impracticability of finding out the ultimate single cadaver fixation and preservative solution workable in all possible scenarios. A comprehensive compilation of different preservation solutions ideal for specific surgical skill training model specimen preparation could be used as guide while planning for cadaveric training workshops.

CONCLUSION

Each of these different methods reviewed in this study exhibited its inherent pros and cons. The responsibility of weighing the benefits of one particular embalming technique over another and addressing its possible health hazards to the handling personnel lies with the expert anatomist. The choice of fixative finally depends upon the consequent purpose for which the preservation was done for that cadaver specifically.

REFERENCES

1. Abdel-Maksoud G, El-Amin AR. A review on the materials used during the mummification processes in ancient Egypt. Mediterranean Archaeology and Archaeometry. 2011;11:129–50.
2. Ajmani M.L. Embalming: Principles and Legal aspects. 1st Ed. Jaypee Brothers Medical Publishers, USA 2008
3. Reznick RK, MacRae H. Teaching surgical skills-changes in the wind. N Engl J Med. 2006;355: 2664–9.
4. Oxentenko AS, Ebbert JO, Ward LE, et al. A multidimensional training model specimen preparation could be used as guide while planning for cadaveric training workshops.
5. Groscurth P, Eggli P, Kapfhammer J, et al. Gross anatomy in the surgical dissecting laboratory. Proc Soc Exp Biol Med. 1997;215:363–5.
6. Macdonald GJ, MacGregor DB. Procedures for embalming cadavers for the dissecting laboratory. Proc Soc Exp Biol Med. 1997;215:363–5.
7. Swenberg JA, Moeller BC, Lu K, Rager JE, Fry RC, Starr TB. Occupational exposure to formaldehyde and alterations in lymphocyte subsets. Am J Ind Med. 2013;56:252–7.
8.inoxyme AM, Axwiog O. EXCRUCIATING EFFECT OF FORMALDEHYDE EXPOSURE TO STUDENTS IN MUSSANAH MEDICAL UNIVERSITY. Int J Occup Environ Med. 2012;3:92–5.
9. Goldstein BD. Hematological and toxicological evaluation of formaldehyde as a potential cause of human leukemia. Hum Exp Toxicol. 2011;30:725–35.
10. Costa S, Costa C, Madureira J, et al. Occupational exposure to formaldehyde and early biomarkers of cancer risk, immunotoxicity and susceptibility. Environ Res. 2019;179:108-740.
11. Humans IWG on the E of CR to. Formaldehyde, 2-Butoxyethanol and 1-tert-Butoxypropan-2-ol. International Agency for Research on Cancer; 2006
12. Brenner E. Human body preservation – old and new techniques. J Anat. 2014;224:316–44.
13. Drsno B, Zubierter T, Gelmetti C, et al. Safety review of phenoxethanol when used as a preservative in cosmetics. J Eur Acad Dermatol Venereol. 2019;33:15–24.
14. Eisma R, Lamb C, Soames RW. From formalin to Thiel embalming: What changes? One anatomy department’s experiences. Clin Anat. 2013;26:564–71.
15. Froligh KW, Andersen LM, Knutsen A, et al. Phenoxethanol as a nontoxic substitute for formaldehyde in long-term preservation of human anatomical specimens for dissection and demonstration purposes. Anat Rec. 1984;208:271–8.
16. Phenoxetol as a formaldehyde-removing agent for long-term preservation: our experience. European Journal of Anatomy. 2020;18:267–72.
17. Kumar R, Verma RK, Dixit RK, et al. Embalming Solutions and their Adverse Effects: An Update. Asian Journal of Pharmaceutical and Health Sciences. 2013;3:735-738
18. Ali J, Shelton JD. Review of fixation techniques for the cryopreservation of embryos. J Reprod Fertil. 1993;99:471–7.
19. Fabbr C, Porcu E, Marsella T, et al. Human oocyte cryopreservation: new perspectives regarding oocyte survival. Hum Reprod. 2001;16:411–6.
20. Bilge O, Celik S. Cadaver embalming fluid for surgical training courses: modified Larsson solution. Surg Radiol Anat. 2017;39:1263–72.
21. Ottone NE, Vargas CA, Fuentes R, et al. Walter Thiel’s Embalming Method: Review of Solutions and Applications in Different Fields of Biomedical Research. Int J of Morph. 2016;34:1442–54.
22. Wolff KD, Kesting M, Mücke T, et al. Thiel embalming technique: a valuable method for microvascular exercise and teaching of flap raising. Microsurgery. 2008;28:273–8.
23. Holzé F, Franz E-P, Lehmbrock J, et al. Thiel embalming technique: a valuable method for teaching oral surgery and implantology. Clin Implant Dent Relat Res. 2012;14:121–6.
24. Hunter A, Eisma R, Lamb C. Thiel embalming fluid—a new way to revive formalin-fixed cadaveric specimens. Clin Anat. 2014;27:853–5.
25. Cabello R, González C, Quíciós C, et al. An experimental model for training in renal transplantation surgery with human cadavers preserved using W. Thiel’s embalming technique. J Surg Educ. 2015;72:192–7.
26. Willaert W, Tozzi F, Van Hoof T, et al. Life-like Vascular Reperfusion of a Thiel-Embalmled Pig Model and Evaluation as a Surgical Training Tool. Eur Surg Res. 2016;56:97–108.
27. Kerckaert I, Van Hoof T, Pattyn P, et al. Centre for Anatomy and Invasive Techniques. Anatomy. 2008;2:28–33.
28. Walther Thiel Method for the Preservation of Corpses with Maintenance of the main physical properties of Vivo. 2020;26.
29. Eisma R, Lamb C, Soames RW. From formalin to Thiel embalming: What changes? One anatomy department’s experiences. Clin Anat. 2013;26:564–71.
30. Hayashi S, Homma H, Naito M, et al. Saturated salt solution method: a useful cadaver embalming for surgical skills training. Medicine (Baltimore). 2014;93:196.
31. Hammer N, Schröder C, Schleifenbaum S. On the suitability of Thiel-fixed samples for biomechanical purposes: Critical considerations on the articles of Liao et al. Clin Anat. 2016;29:424–5.
32. Eisma R, Lamb C, Soames RW. From formalin to Thiel embalming: What changes? One anatomy department’s experiences. Clin Anat. 2013;26:564–71.
33. McDougall S, Soames R, Felts P. Thiel embalming: Quantifying histological changes in skeletal muscle and tendon and investigating the role of boric acid. Clin Anat. 2019;
34. Lásdó GJ, Sáfía Z, Nemeskérí A, et al. Human cadavers preserved using Thiel’s method for the teaching of fibreoptically-guided intubation of the trachea: a laboratory investigation. Anaesthesia. 2018;73:65–70.
35. Eisma R, Mahendran S, Majumdar S, et al. A comparison of Thiel and formalin embalmed cadavers for thyroid surgery training. Surgeon. 2011;9:142–6.
36. Giger U, Frézard I, Hültiger A, et al. Laparoscopic training on Thiel human cadavers: a model to teach advanced laparoscopic procedures. Surg Endosc. 2008;22:901–6.
37. Eisma R, Wilkinson T. From "silent teachers" to models. PLoS Biol. 2014;12:1001971.
38. Eljamel S, Volovick A, Saliev T, et al. Evaluation of Thiel cadaveric model for MRI-guided stereotactic procedures in neurosurgery. Surg Neurol Int. 2014;5:404-409.
39. Mains E, Tang B, Golabek T, et al. Ureterorenoscopy training on cadavers embalmed by Thiel’s method: simulation or a further step towards reality? Initial report. Cent European J Urol. 2017;70:81–7.
40. Eljamel S, Volovick A, Saliev T, et al. Evaluation of Thiel cadaveric model for MRI-guided stereotactic procedures in neurosurgery. Surg Neurol Int. 2014;5:404-409.
41. Liao PY, Wang ZG. Thiel-embalming technique: investigation of possible modification in embalming tissue as evaluation model for radiofrequency ablation. J Biomed Res. 2019;
42. Burns DM, Bell I, Katchky R, et al. Saturated Salt Solution Cadaver-Embalming Method Improves Orthopaedic Surgical Skills Training. J Bone Joint Surg Am. 2018;100-104.
43. Coleman R, Kogan I. An improved low-formaldehyde embalming fluid to preserve cadavers for anatomy teaching. J Anat. 1998;192:443–6.
44. Homma H, Oda J, Sano H, et al. Advanced cadaver-based educational seminar for trauma surgery using saturated salt solution-embalmed cadavers. Acute Med Surg. 2019;6:123-30.
45. Gosomji IJ, Omirinde JO, Hena SA, et al. Saturated Salt Solution an Alternative Reagent in Reducing Formaldehyde Concentration in Embalming. MOJ Anat & Physiol. 2018;5:205–7.
46. Hammer N, Löffler S, Feja C, et al. Substitution of formaldehyde in cross anatomy is possible. J Natl Cancer Inst. 2011;103:610–1.
47. Hammer N, Löffler S, Feja C, et al. Ethanol-glycerin fixation with thymol conservation: a potential alternative to formaldehyde and phenol embalming. Anat Sci Educ. 2012;5:225–33.
48. Hammer N, Löffler S, Bechmann I, et al. Comparison of modified Thiel embalming and ethanol-glycerin fixation in an anatomy environment: Potentials and limitations of two complementary techniques. Anat Sci Educ. 2015;8:74–85.
49. Abdul Momen A, Al-Hayani, Raid M, Hamdy, et al. Shellac: A nontoxic preservative for Human embalming techniques. Journal of Animal and Veterinary Advances. 2011;10(12):1561–7.
50. Goyri-O’Neill J, Pais D, Freire de Andrade F, et al. Improvement of the embalming perfusion method: the innovation and the results by light and scanning electron microscopy. Acta Med Port. 2013;26:188–94.
51. Natekar PE, Desouza FM. A New Embalming Fluid for Preserving Cadavers. 2012;1(2):5.
52. Maruyama K, Yokoi H, Nagase M, et al. Usefulness of N-vinyl-2-pyrrolidone Embalming for Endoscopic Transnasal Skull Base Approach in Cadaver Dissection. Neurol Med Chir. 2019;59:379–83.
53. Hanzuka Y, Nagase M, Takashino S, et al. A new substitute for formalin: Application to embalming cadavers. Clin Anat. 2018;31(1):90–8.
54. van Emden MW, Geurts JJ, Schober P, et al. Comparison of a Novel Cadaver Model (Fix for Life) With the Formalin-Fixed Cadaver and Manikin Model for Suitability and Realism in Airway Management Training. Anesth Analg. 2018;127(4):914–9.