REVIEW

Human Preservation Techniques in Anatomy:

A 21st Century Medical Education Perspective

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Anatomy is the cornerstone of education for healthcare professionals with the use of human material providing an excellent teaching tool in the modern curricula. The ability and quality of preservation of human remains has enabled such use. The introduction of formaldehyde as a preservative in 1893 was an important step in the history of preservation. With the European Union directive on the use of formaldehyde and its expected banning, anatomists are trying to find a more convenient and safe substitute. In this review, we compare the different techniques used based on the need for embalming, fixative used, period of preservation and the features of the embalmed specimen. The fact that embalming is used in different disciplines, multiple purposes and described in different languages has led to the development of ambiguous interchangeable terminology. Overall, there is a lack of information specifically classifying, listing and comparing different embalming techniques, and this may be due to the fact that no internationally recognized experimental standards are adhered to in this field. Anatomists strive to find an embalming technique that allows the preserved specimen to accurately resemble the living tissue, preserve the body for a long period of time and reduces health risk concerns related to working with cadavers. There is a need for embalming to shift to an independent modern day science with well-founded research at the heart of it. While this may take time and agreement across nations, we feel that this review adds to the literature to provide a variety of different methods that can be employed for human tissue preservation depending on the desired outcome. Clin. Anat. 28:725-734, 2015. @ 2015 Wiley Periodicals, Inc.

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INTRODUCTION

For more than 3,000 years human beings have tried to stop after-death body decay to preserve the mortal frame for the afterlife or reanimation (Weiglein, 2002). For example, the mummification performed by the ancient Egyptians and cryopreservation in the 20th century (Jones, 1997). Embalming is a chemical process that is used to preserve and sanitize the human body after death (Bradbury and Hoshino, 1978). The practice of embalming in more recent times has been performed to keep the dead body in good condition until after the funeral which is believed to have started in 1861 during the American civil war (Ezugworie et al., 2008). Injecting fluids into the

body's vessels as part of the embalming process only came after William Harvey described the blood circulation (Aird, 2011) The basic constituents of the various embalming formulas are divided into six general chemical groups: preservative or fixative, disinfectant, modifying agent (buffer, anticoagulant, and surfactant,

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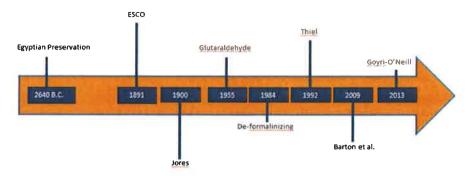


Fig. 1. A timeline of different preservation techniques from the ancient Egyptian to the current embalming technique. (ESCO=The Embalmet's Supply Company, Ontario). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

humectant), dyes, perfuming agents and diluents (Mayer, 2011).

The main concern of the embalmer for funeral reasons is the outer appearance of the body, the preservation of tissue and the disinfection of the body to reduce any potential infection risks (Davidson and Benjamin, 2006; Trompette and Lemonnier, 2009). Embalmers aim to disinfect the body to decrease any chances of infection to the general public that are viewing the body and to maintain a healthy working environment (Davidson and Benjamin, Embalming solutions are tested for their ability to kill bacteria such as Staphylococcus aureus, Pseudomonas aeruginosa, and Proteus vulgaris (Woodburne and Lawrence, 1952). The study of human morphology also requires the preservation of the body to allow anatomical investigation over an extended period of time, or the use of fresh cadavers for a shorter investigation. Human cadavers could be a source of infection for both students and teachers when used for dissection, therefore, embalming is used to reduce or eliminate that risk (Shoja et al., 2013). The sourcing of cadavers has gone through many challenges and phases. It has also passed through periods of changing legislation, where challenges have originated from religious and cultural issues, or other personal considerations (Anyanwu et al., 2011). Different sources and methods of cadaver acquisition have been used in anatomical history. An example of these sources include bodies of gibbeted victims and bodies of those executed by other means, stolen bodies from graveyards, unclaimed bodies, criminals that died in battle or in prison, bodies of victims of murder, and most recently body donation (Anyanwu et al., 2011).

One of the practical methods used to teach human anatomy is by dissecting a human cadaver. Dissection has been described by some anatomists as "a mainstay of properly rigorous basic science training" (McLachlan et al., 2004). It is widely believed that the process of dissection adds a three dimensional view to the students' knowledge, and reinforces concepts introduced in lectures and tutorials (Turner et al., 2005).

Formaldehyde was first discovered by August Wilhelm von Hofmann in 1863 and its introduction as a

fixative in 1893 was an important step in preservation (Blum, 1893; Binawara et al., 2010). Figure 1 is a timeline of different preservation techniques, from ancient Egyptian to currently used methods, according to their dates of usage. Formaldehyde is used to preserve human tissue, and also used to preserve zoological and entomological specimens (Fox et al., 1985). One of the main functions of formaldehyde is the cross-linking of tissue constituents proteins, which make the tissue less accessible to microorganisms. preventing its decay (Fox et al., 1985; Mason and O'Leary, 1991; Titford, 2012). Vapors secreted from formalin-embalmed cadavers have been suggested to be carcinogenic even with the use of personal protective equipment in the gross anatomy laboratory (Hubbell et al., 2002; Guo et al., 2012). Formalin embalmed cadavers do not exhibit many of the qualities of living organs, among which are color, softness, pliability, the pulsatile nature of the heart and arteries, and the expansion of aerated lungs (Hubbell et al., 2002).

An extensive literature search was performed to document the different embalming techniques that are used to preserve human cadavers. The aim of this review is to focus on the preservation of a full human cadaver. The main criteria used to filter the results can be divided into four categories. The first criterion is whether the solution described in the article has been used or it could potentially be used on a human cadaver. The second criterion is the fixative or preservative used in the solution (formalin vs.non formalin). Since the value of the fixative can be assessed in relation to the efficacy of the method of delivery and subsequent storage practices, these details where mentioned when available but were not used as a criterion as they are not available for all techniques. The third criterion is the preservation period under which the cadaver could be used before decomposition starts. The fourth is the quality of tissue and organs of the preserved human cadaver, though there are no clear criteria on how to decide whether the embalmed body is soft or hard-fixed.

Some terms used for describing preservation techniques are considered to be ambiguous; therefore

there was a need to define some terms that will be used. The term "fresh cadaver" describes a human cadaver that has not been chemically treated (embalmed). When the embalming solution produces a cadaver with joint flexibility less than that of a fresh cadaver and with hard internal organs, this cadaver is described as "hard fixed." "Soft preserved cadaver" is used to describe cadavers that have equal or more joint flexibility than that of a fresh cadaver and with soft internal organs, "Formalin based solution" is used to describe solutions containing more than 0% formaldehyde while solutions containing 0% formaldehyde are described as 'Non-formalin based solution'.

FORMALIN BASED SOLUTIONS

Hard-Fixed

Kaiserling, Jores, Tutsch, et al. and Neumann is a group of the oldest embalming solutions, named after their authors, which use formaldehyde as the fixative in their formula (Kaiserling, 1900; Jores, 1913; Tutsch et al., 1971; Neumann, 1974), Jores embalming technique is still being used at the University of Fribourg, Switzerland where it results in hard-fixation (Luis Filgueira- University of Fribourg, personal communication). Bacterial and fungal tests were performed on two formalin embalmed cadavers. Cadavers showed positive growth of microorganisms before embalming while 14 days after embalming tests showed that formaldehyde was able to kill the bacteria and fungus present in the pharynx, rectum, pleural fluids, and ascites (Hayashi et al., 2014).

A relatively recent French study whereby 109 anatomy centers across the five continents described the embalming technique that they use, reported that formaldehyde was used in almost 87% of centers, and consistently across the five continents: 84% in Europe, 87% in America, and 100% in the other three continents (Benkhadra et al., 2011). Our department has used a formalin based hard fixing recipe to embalm donated cadavers along with fresh frozen cadavers for surgical training for many years now. Though the main embalming technique used in the department is formalin based, we are currently looking at different embalming techniques as part of a research project and the first author of this article has experience in soft preservation.

Genelyn is a commercial product produced by Genelyn Pty. Ltd, Australia. Genelyn embalmed cadavers are generally less flexible compared to fresh cadavers (Jaung et al., 2011). The material safety data sheet (MSDS) provided by the company indicates that formaldehyde is an active ingredient of this solution (Genelyn, 2010). Genelyn embalmed cadavers are also noted to be stiffer and more brittle compared to unembalmed tissue (Norton-old et al., 2013). Ideally the cadaver would be placed in a flexed position for clinical training, but due to the lack of flexibility genelyn embalmed cadaver used in prone position (Belavy et al., 2011). It should be noted that there is only limited available literature that describes the quality of tissue in cadavers embalmed by Genelyn solution (Belavy et al., 2011; Jaung et al., 2011; Norton-old et al., 2013).

Soft-Fixed

It is now over 20 years since Walter Thiel developed a novel preservation method that focused on tissue color preservation that set the basis for a new photographic atlas of practical anatomy (Thiel, 1992). Several articles describe how well the texture and color is preserved in cadavers embalmed with Thiel technique (Benkhadra et al., 2011; Guo et al., 2012; Prasad Rai et al., 2012; Desroches et al., 2013). Cadavers embalmed by Thiel technique are preserved for over a year after being taken out of the tank of embalming fluid. This embalming technique includes a small percentage of formaldehyde compared to the commonly used formalin based recipes along with other salts such as ammonium nitrate, potassium nitrate and sodium sulfite (Eisma et al., 2013).

On a different note, a light embalming technique is used in the surgical fresh tissue dissection laboratory at the University of Louisville, Kentucky. When dissection starts, cadavers embalmed with this technique can be used for 2 weeks or can be stored at 4°C for 6 weeks. Cadavers embalmed with this technique produced tissue close to that found in the living body both in color and texture, and the abdominal cavity did not have an offensive odor. The embalming fluids used in this technique are weaker than the commonly used ones and smaller volumes are introduced without any pressure. No antimicrobial testing has been performed on this technique, but no known instance of transmission of bacterial or viral disease has been recorded (Anderson, 2006).

Deformalinizing Solutions

Phenoxyethanol is a non-toxic, lightly scented, relatively inexpensive, non-flammable, slow to evaporate, effective antimicrobial, excellent tissue preservative and softener that could be used to remove formaldehyde from fixed tissue. A formaldehyde based embalming technique was published in 1984 that includes the use of phenoxyethanol for post-fixation, long term preservation of embalmed cadavers (Frølich et al., 1984). Wineski and English (1989) described this technique as lengthy and cumbersome, therefore, they suggested a modified technique which is significantly shorter as it eliminates the phenoxyethanol immersion period. They described their technique as safer since the process utilize significantly lower volumes and concentrations of formaldehyde (Wineski and English, 1989).

NON-FORMALIN BASED SOLUTIONS

Hard-Fixed

None of the non-formalin based solutions can be described as hard fixed.

Solutions Used for Soft-Preservation

There have been some efforts made to develop soft-preserving approaches with non-formalin-based solutions. A soft preserving technique has been described to embalm cadavers for postgraduate courses in order to more closely reflect the appearance and feel of the human body at surgery (Barton et al., 2009). The embalming chemicals consist of a mixture of alcohol, water, glycerol, and phenol (Barton et al., 2009). This embalming solution produces a cadaver that can be used for a period of about 6 months, when stored (Prof. Ceri Davies- Imperial Col-

lege London, Personal communication).

Different solutions provided by The Embalmer's Supply Company, Ontario (ESCO), Canada have been used to embalm donated human cadavers (Messmer et al., 2010). The ESCO EPIC Conditioner is used as a humectant, plasticizer, preservative, and anti-dehydrant, and the main ingredient according to the material safety data sheet of the product provided by the company is methanol (8.6%) (ESCO, 2012). The other solution described by the article is the ESCO anticoagulant softener, which according to the material safety data sheet of the product provided by the company the ingredients name are methanol, ethylene glycol, and EDTA Na₃ (ESCO, 2012). It was proposed that cadavers would stay intact for 30 days after being thawed, or 45 days if they were never stored below zero. The removal of blood decrease the growth of bacteria and unlike other preservation techniques, mold growth was not observed during the period of which the cadaver was sued (Messmer et al., 2010).

Aliphatic alcohols, diethylene glycol and monoethylene glycol (90:10), were used to prepare an embalming solution at the Universidade Nova de Lisboa, Lisbon, Portugal. Both monoethylene glycol and diethylglycol are toxic chemicals (Goyri-O'Neill et al., 2013). However, they do not produce toxic vapors at room temperature. Cadavers embalmed with this solution showed no increase in skin resistance, detachment of skin layers, significant changes in coloration and joints remained movable without significant changes of passive range of motion (Goyri-O'Neill et al., 2013). This quality of tissue was preserved for a period between 6 months to 1 year after embalming (Goyri-O'Neill et al., 2013).

An embalming solution has been published in 2011 using 2-brono-2-nitropropane-1,3-diol as its main constituent. Bronopol is a biocide which presents little or no risk to human health and it is not volatile at room temperature. Practitioners who carried out dissection on a cadaver embalmed with a bronopol based solution considered this product far superior to formaldehyde as the later led to very rapid dehydration making the skin and tissue very hard (Dissard et al., 2009). Full body dissection performed 14 and 16 days after death and dissection of the heart after 8 months showed that the cadaver was well preserved (Dissard et al., 2009).

Ethanol-Glycerin fixation and Thymol conservation were used by Hammer et al. (2012) to embalm a human cadaver. The tissue remained close to its natural state especially nerves and arteries (Hammer et al., 2012). This embalming solution produces a

cadaver with flexible muscles and hollow organs such as the gastrointestinal tract and urinary bladder. Thymol is known to be an effective bactericidal and fungicidal and therefore no bacteria was noticeable during the dissection course (Hammer et al., 2012).

Glutaraldehyde was first used as a fixative for embalming in 1955 (Tolhurst and Hart, 1990). Several embalming solutions were patented with glutaraldehyde as its main ingredient in 1962, 1975, and 1998 (Campbell and Margrave, 1998). Human tissue treated with the solution produced by Campbell and Margrave exhibit good color retention in muscles, has no odor, and is easy to work with (Campbell and Margrave, 1998). Unlike formaldehyde, less moisture is taken from tissues as a result of the chemical reaction between glutaraldehyde and proteins hence resulting in more flexible tissue (Campbell and Margrave, 1998; Mayer, 2011). Tolhurst and Hart (1990) described an embalming solution with gultaraldehyde as its main ingredient where the cadaver retained a life-like texture for 6 weeks if they were stored in a refrigerated unit (Tolhurst and Hart, 1990). Glutaraldehyde has also been used in whole-body animal fixation for electron microscopy. Perfusing 1-3% of glutaraldehyde produced a hard fixed specimen with immobile limbs (Nawroth et al., 1986).

Potential Solutions for Soft-Preservation

Polyhexamethyleneguanidine hydrochloride (PHMGH) is a non-volatile substance without any odor and considered as a substance of low toxicity. An attempt to use PHMGH as a preservative has been reported in 2009 (Ryabinin et al., 2009). A study in 2011 used PHMGH to fix a 12-day chick embryo and other human organs (Anichkov et al., 2011). The appearance of organs fixed with PHMGH was natural and showed no visual difference compared with native autopsy material, while fragments of tissue/organs showed a soft elastic consistency (Anichkov et al., 2011).The fixed chick embryo and human organs retained the characteristics specified above for a year and a half (Anichkov et al., 2011).

Ionic liquids are a class of organic salts that are liquid at room temperature. They can be recycled and reused; therefore they were called green solvents. A study in 2003 used ionic liquids for embalming where animal tissues kept a pleasant smell, no color change and no significant shrinkage or dehydration of tissue over a period of 2 years (Majewski et al., 2003).

PATENTS

The patent literature also reveals a number of novel potential solutions. US patent 3,264,182 used 2-paradioxanon (δ-lactones as the active preservative ingredient) to preserve specimens of the animal kingdom (which could be human tissue or cadaver). The patent describes several methods to treat the specimen such as immersion, injection, or infusion. The inventors describe the specimen as flexible and life-like post embalming. Different concentrations of the embalming material could be used for different needs, ranging

TABLE 1. Comparison of Different Embalming Techniques

Technique	Use on human cadaver	Fixative used	Period of preservation ^a	Quality of tissue	References
Genelyn	Yes	Formalin based	N/A	Harder than Fresh	(Jaung et al., 2011; Norton-old et al., 2013)
Thiel	Yes	Formalin based (low)	Over a Year	Similar to Fresh	(Thiel, 1992)
Anderson	Yes	Formalin based (diluted)	Two weeks	Similar to Fresh	(Anderson, 2006)
Førlich	Yes	Formalin based (buffered)	N/A	N/A	(Frølich et al., 1984)
St. George's	Yes	Alcohol, phenol and glycerol	Six months	Similar to Fresh	(Barton et al., 2009)
ESCO	Yes	ESCO Epic conditioner and ESCO anticoagulant softener	30 days	Similar to Fresh	(Messmer et al., 2010)
Universidade Nova de Lisboa	Yes	Aliphatic alcohols, Diethylene glycol and Monoethylene glycol	Six months to a Year	Similar to Fresh	(Goyri-O'Neill et al., 2013)
Bronopol	Yes	Bronopol	Not clear	Similar to Fresh	(Dissard et al., 2009)
Ethanol-Glycerine	Yes	Ethanol, glycerine and Thymol	One year	Similar to Fresh	(Hammer et al., 2012)
Glutaraldehyde	Yes	Glutaraldehyde	Six weeks	Similar to Fresh	(Campbell and Margrave, 1998; Tolhurst and Hart, 1990)
PHMGH	No	Polyhexamethyleneguanidine hydrochloride	N/A	Similar to Fresh	(Anichkov et al., 2011)
Ionic Liquids	No	Ionic liquids	Two years	Similar to Fresh	(Majewski et al., 2003)
δ-lactones	No	2-para-dioxanon	N/A	Similar to Fresh	(Langner and Jackson, 1966)

^aThe period of preservation could be limited by the legislations governing different anatomy departments as different countries allow retaining body parts for different periods of time.

from specimen in permanent exhibitions to specimen used for weeks in dissection based courses (Langner and Jackson, 1966).

A vegetable based water solution polymer, PolyOxy-Methylene dialkyl ether, quaternary ammonium compound and propolis were used as active ingredients in different embalming solution. These solutions were patented in US80152777B2 (2011), US0020910A1 (2012), US20120297593, and EP1127490A1, respectively (Clerc and Sigoure, 2001; Barrow, 2011; Berry and Thomas, 2012; Sigoure et al., 2012).

Table 1 summarizes the different techniques based on the different criteria used for comparison. Trying to summarize this type of information into one table is difficult as different standards are used in different articles. There is no unified definition for the term fixative, it was challenging to pick one chemical out of a mixture. Also authors did not specify the conditions under which the cadavers are stored; therefore it is difficult to compare the preservation period of each solution. The need for an embalmed cadaver that could accurately mimic a fresh cadaver and especially that there are no criteria for comparison makes it impossible to compare the quality of tissue as authors will tend to describe their techniques as fresh-like.

EDUCATION VERSUS FUNERAL INDUSTRY

Recent attention has also been given to comparing different embalming techniques for both the educational and the funeral industry needs (Brenner, 2014).

The use of embalming in both fields led to the use of interchangeable terminology which made it the work problematic to categorize different embalming techniques and solutions. Many of the older embalming articles were written in German and its translation to English might be interpreted differently (Thiel, 1992). For example, Langner and Jackson described formal-dehyde as a preservative while Hammer et al. described it as a fixative and they both were describing the same characteristics of this chemical (Langner and Jackson, 1966; Hammer et al., 2012).

The need of embalming in the funeral industry is for aesthetic reasons for certain parts of the body over a short period of time and reducing any potential health risks. While the need of embalming in education/research setting is to prevent the decomposition of the entire body over a longer period of time retaining features of unembalmed cadaver.

WHAT DO ANATOMISTS CONSIDER?

Knowing that embalming is performed to prevent the decomposition of the human body, the primary focus is to reduce/eliminate the presence of pathogens that could increase the risk of infection. Other factors that anatomists would consider when comparing different embalming techniques are fixative used, decomposition rate and quality of tissue.

Fixative

It is important to emphasis the key elements that anatomists focus on when considering an embalming

solution. From the literature, it is difficult to identify the type of fixative used in every solution because of the ambiguous terminology used and especially that there is no universal definition for this term. Knowing the type of fixative used in an embalming solution helps in analyzing its effect on tissue on both macro and microscopic levels. Moreover, the use of certain fixatives in some countries might be illegal as it could be banned by their regulatory bodies. Formaldehyde is being used by the majority of embalmers across the globe, although there are some threats to ban its use in Europe (Brenner, 2014). In order to focus on other alternatives, we have decided to focus and divide our results into formalin and non-formalin based solutions.

Quality of Tissue

Another key element is the quality of tissue and organs preserved in the embalmed cadaver. Before the widespread use of embalming, fresh cadavers were used to learn about the human body (Silverman, 1991). Formaldehyde causes the discoloration of tissue, stiffness of joints and dehydration of organs/tissue, therefore, practically making it incomparable to the previous state. Despite this, formalin embalmed cadavers are still a good teaching tool for learning basic human anatomy. Surgery is the one specialty that brings anatomy to life and brings the surgeons back to the dissecting room; they questioned how realistic the embalmed human cadavers are. This come back has raised questions among anatomists whether the anatomy being taught accurately represent the human body, especially for medical students. A lot of research is being carried out to find an embalming solution that produces a cadaver as 'lifelike' as possible. This is another example of an ambiguous terms being used, where the ideal embalming solution can keep its features close to an unembalmed cadaver but not a life-like state as that would require the circulation of blood (which is currently being investigated). We have decided to use the term fresh cadaver to describe unemablmed cadaver. "Soft-fix" or "soft preserved" is another term as Barton et al. discussed and that is based on the definition of the term fixative. With the need of a new embalming technique, all new embalming solutions claim to produce a freshlike cadaver. The lack of quantitative criteria to assess these claims, make it difficult to compare the quality of tissue and organs embalmed with these solutions.

Decomposition Rate

The third key element is the period in which the embalming solution can protect the human cadaver from decomposition. In the funeral industry embalming for a week would be the ideal scenario, while in an academic/research setting the required period is over 8–12 months. Even the need in an academic/research setting might be different, as using a cadaver for a dissection course may require its use for a longer period compared to a series of surgical training workshops. Decomposition rate is another area where

there is lack of quantitative methods to measure the decomposition of an embalmed cadaver. The currently used method is by examining the cadaver over a period of time and detecting any odors of active decomposition (Anderson, 2006; Messmer et al., 2010).

Differences between Formalin Solutions

Many embalming solutions that use formaldehyde as their fixing agent are available and with a slight change of percentages, it could be named and claimed as a new solution. Therefore, we have decided not to focus on the formalin-based solutions that produce a hard fixed cadaver.

Genelyn

Genelyn solution is a commercial product manufactured in Australia. In the past few years, embalmers have noticed a difference in tissue and joints of cadavers embalmed with this technique compared to the regular formalin based solution. These claims come in contrast to the majority of published papers (Belavy et al., 2011; Jaung et al., 2011; Norton-old et al., 2013) that describe Genelyn embalmed cadavers as stiff and rigid.

Thiel

Over the past number of years, Thiel embalming solution has gained increasing attention even though the first article was published in 1992. One of reasons could be due to the fact that the original article was published in German. Although cadavers embalmed by this technique have shown features close to fresh cadavers compared to formalin embalmed cadavers (Jaung et al., 2011; Eisma et al., 2013), others have criticized some areas such as tendons (Fessel et al., 2011) muscles (Benkhadra et al., 2011) and bones (Unger et al., 2010). On the technical level, the Thiel embalming technique is relatively expensive, time consuming and difficult to perform (Wolff et al., 2008; Hammer et al., 2012). Moreover, Janczyk et al. (2011) describe some of the chemical used to prepare the Thiel solution as poisonous, very flammable, explosive, extremely hazardous to health and environmentally unfriendly. To reduce the formation of fur-anes and dioxins, Thiel cadavers must be cremated at high a temperature which is considered to be a disadvantage of this solution (Janczyk et al., 2011). Thiel embalmed cadavers can be used to teach the gross anatomy of the body as it mimics the human body, while it might not be ideal to use for some surgical training. More research is needed research should be done on the chemicals secreted post cremation.

Other Solutions

In addition to Genylyn and Thiel, the use of high concentrations of formaldehyde in other solutions produced a rigid cadaver, the introduction of salts along with an extended period of immersion produced a

flexible cadaver with a long life time, and the use of low formaldehyde concentrations along with salts produced a flexible cadaver preserved for a shorter period of time. In 2006, Anderson described the use of diluted Dodge solutions (formalin based) that would produce a soft cadaver that could be used for a period of 2 weeks. Da Silva et al. (2004) described another embalming solution based on formalin and other salts that embalmed a dog cadaver for 2 weeks where desquamation of the abdominal and inquinal regions was observed afterwards (Da Silva et al., 2004). The solution was based on modifying Larson solution and it was compared to Klotz solution. Another solution that produced a relatively soft and plastic cadaver with joints that move with ease was introduced by Woodburne and Lawrence (1952), and this solution was formalin based with other chemicals. Richins et al. (1963) suggested the use of pyrophosphate and magnesium chloride to the formalin based solution to produce a cadaver with pliable muscles and freely movable joints (Woodburne and Lawrence, 1952; Richins et al., 1963).

In an effort to decrease the vapors of formaldehyde in the dissecting room, phenoxyethanol has been introduced as part of the embalming process. It is not a fixative, therefore, it does not replace formaldehyde in the embalming process. Although Wineski and English measured the vapors of formaldehyde after immersion in phenoxyethanol, they did not measure the vapors before immersion. They assumed that it has decreased based on data from other published articles about the vapors secreted from formalin

embalmed cadavers (Pabst, 1987).

Differences between Non-Formalin Embalming Solutions

The need for a formalin-free and fresh-like cadaver led anatomist research for a new embalming technique. With the lack of criteria on whether an embalmed cadaver possess the features of a fresh cadaver and as researchers are trying to avoid formaldehyde, no formalin-free solution was found in the literature that describe the produced cadaver as hard fix. Many of the new embalming solutions are not tested by anatomists, which led us to divide the results into solutions used for soft preservation and potential solutions. Solutions that have been used for soft preservation are the ones where dissection has been conducted on the cadaver embalmed with this solution.

With non-formalin based embalming solutions that produce a fresh like cadaver, the challenge is the period which the solution would protect the cadaver from decomposition. There is a need to extend the preservation period of a cadaver in order to maximize the number of beneficiaries, especially with the decline of body donation and shortage in anatomical specimens (Gunderman, 2008). Though the embalming solution described by Goyri-O'Neil et al. did not mention the condition of the internal organs, this solution preserved bodies for a period of 1 year. Hammer et al. described an ethanol-glycerin embalming solution that would preserve a human cadaver for at least 12 months. Barton

et al. described an embalming solution that softly preserved the cadaver for a period of 6 months. The solution used at the Duke human fresh tissue lab (ESCO solution) and by Tolhurst and Hart preserves the body for 6 weeks (Barton et al., 2009; Hammer et al., 2012; Goyri-O'Neill et al., 2013)

Bronopol is an active ingredient in the embalmina solution patented by Dissard et al. (2009) and is an active substance in the review program published by the European Commission under the 'Guidance document on the evaluation of efficacy of embalming products (PT22) (Dissard et al., 2009; European-Commision, 2013). Bronopol can decompose into nitrite which in turn and in the presence of certain secondary and tertiary amine and amides can form nitrosamines which may be carcinogenic (Bryce et al., 1978). Other solutions containing Shellac and Nirite pickling salts were described by Al-Hayani et al. (2011) and Janczyk et al. (2011), respectively, but because of the unrealistic color of the cadavers produced they were not mentioned in this review (Al-Hayani et al., 2011; Janczyk et al., 2011).

Un-experimented soft preserving solutions have great potential especially those that have been tested as they have proven to be successful for embalming of animal specimen for a long period of time. There is a need to try these embalming solutions on human cadavers and test the quality of tissue preserved. On the other hand, there this is a group of embalming solutions that could be of potential use in funeral industry as they can preserve the body for a shorter period of time. Table 2 summarizes the advantages and the disadvantages of the different techniques.

Future Directions

Scientists are trying to develop an ideal embalming solution to preserve the human body. The ideal embalming solution would preserve the body in conditions comparable to that of an un-embalmed cadaver. The chemicals used in this solution must be nonhazardous to eliminate any health risks that students, academics and researchers might encounter when dealing with the embalmed cadaver. The embalmed cadaver should be relatively bacteria free and the embalming solution should protect the body from being a host for any other microorganisms that will speed the decomposition process.

The use of embalming in modern day sciences requires its move from a "word of mouth" practice into a more academic/research based science discipline. Embalming is a very old practice used in different countries and in multiple disciplines, therefore the majority of the documentation is held confidentially in some institutions or published in languages other than English. This has led to the creation of interchangeable ambiguous terminology among funeral embalming, veterinary embalming and human anatomy embalming, therefore, there is a need to classify publications based on their use. The move of embalming from a practice into a science would speed up the process of developing clear scientific terminology.

TABLE 2. Advantages and Disadvantages of Different Embalming Techniques

Technique	Advantages	Disadvantages	References
Genelyn	Claims of flexibility Period of preservation	Formalin based Not enough research	(Jaung et al., 2011)
Thiel	Flexibility Multi-disciplinary use Realistic colors No offensive odor Period of preservation Very low formalin Well researched	Relatively more expensive Complexity of embalming	(Benkhadra et al., 2011; Eisma et al., 2013; Thiel, 1992)
Anderson	Realistic colors and texture	Short preservation period Formalin based Not enough research	(Anderson, 2006)
Førlich	De-formalinizing Relatively flexible	Complexity Formalin based Not commonly used Not enough Research	(Frølich et al., 1984)
St. George's	Flexibility Realistic colors No formalin Quick embalming	Short preservation period Not enough research	(Barton et al., 2009)
ESCO	Flexibility Realistic colors	Short preservation period Not enough research	(Messmer et al., 2010)
Universidade Nova de Lisboa	Relatively flexible No change in Color No formalin	Not enough research	(Goyri-O'Neill et al., 2013)

In the 21st Century, medical schools need an embalming technique that preserves the body in a realistic manner, which requires the development of internationally recognized standards to compare the different embalming techniques. These standards should be based on experimental protocols that would enable anatomists/embalmers to compare the quality of tissue produced by a specific technique and quantitatively asses the decomposition of cadavers.

The turning point in anatomy which was induced by the return of surgeons to the dissecting room for surgical training is remarkable. History records that the use of fresh human cadavers to learn anatomy is critical (Holomanova et al., 2001). With the introduction of formaldehyde anatomists moved away from fresh cadavers into a new product which is not the real body, though formaldehyde provided the advantage of working in a safer environment over a longer period of time. Generation after generation anatomists now have no encounter with fresh human material and are trained and taught using formalin cadavers results in them being unfamiliar to deal with the fresh cadavers. With the invention of soft preserving embalming techniques, anatomists are realizing that this could serve both needs to teach anatomy and provide a cadaver for surgical training. If used for anatomy teaching, there will be a need to develop new teaching material because soft preserved cadavers are different to formalin cadavers (Eisma et al., 2013).

CONCLUSIONS

Donation of the human body is a generous act that anatomists highly regard; therefore, efforts should be made to make the most out of this donation by maximizing the number of users. Finding an embalming technique that preserves the body in a realistic manner, not only serves the reason why anatomy is taught, but also invites clinicians to investigate new techniques. The donated cadaver could be described as a shared resource used by academics, researchers and clinicians. The initial users will include noninvasive clinical skills, which is followed by different beneficiaries such as radiologists, minimal invasive surgeons, equipment testing companies, general/multidisciplinary surgeons and will finally be used for anatomy teaching. The need to define some terms for the purpose of this article is a limitation as some may consider them subjective. The move of embalming into an independent modern day science will encourage more experimental research which will include developing protocols that will assist anatomists/ embalmers to assess the different embalming techniques. Anatomists are trying to intentionally preserve the body in a realistic manner for a long period of time, yet a lot is still to be learnt from the unintentional preserved bodies during history such as the bog bodies that are scattered throughout Scandinavia, United Kingdom, and Ireland (Dell'Amore, 2014).

REFERENCES

Aird WC. 2011. Discovery of the cardiovascular system: From Galen to William Harvey. J Thromb Haemost 9 Suppl 1:118–129.

Al-Hayani AA, Hamdy RM, El-Aziz GSA, Badawoud MH, Aldaqal S, Bedir Y. 2011. Shellac: A non-toxic preservative for human embalming techniques. J Anim Vet Adv 10:1561–1567.

Anderson SD. 2006. Practical light embalming technique for use in the surgical fresh tissue dissection laboratory. Clin Anat 19:8–11.

- Anichkov NM, Danilova IA, Vasiliev OD, Ryabinin IA, Kipenko AV. 2011. Use of a polyguanidine solution for fixing biological and anatomical specimens. Neurosci Behav Physiol 41:18–21.
- Anyanwu GE, Udemezue OO, Obikili EN. 2011. Dark age of sourcing cadavers in developing countries: A Nigerian survey. Clin Anat 24:831–836.
- Barrow DCJ. 2011. Embalming fluid. In: Google Patents.
- Barton DP, Davies DC, Mahadevan V, Dennis L, Adib T, Mudan S, Sohaib A, Ellis H. 2009. Dissection of soft-preserved cadavers in the training of gynaecological oncologists: Report of the first UK workshop. Gynecol Oncol 113:352–356.
- Belavy D, Ruitenberg MJ, Brijball RB. 2011. Feasibility study of realtime three-/four-dimensional ultrasound for epidural catheter insertion. Brit J Anaesth 107:438–445.
- Benkhadra M, Gerard J, Genelot D, Trouilloud P, Girard C, Anderhuber F, Feigl G. 2011. Is Thiel's embalming method widely known? A world survey about its use. Surg Radiol Anat 33:359–363.
- Berry SD, Thomas AJ. 2012. Tissue Preservation Fluid, In: Google Patents.
- Binawara B, Rajnee CS, Mathur K, Sharma H, Goyal K. 2010. Acute effect of formalin on pulmonary function tests in medical students. Pak J Physiol 6:8–10.
- Blum J. 1893. Formol als Konservierungs flüssigkeit. Zool Anz 16: 450–452.
- Bradbury SA, Hoshino K. 1978. An improved embalming procedure for long-lasting preservation of the cadaver for anatomical study. Acta Anat (Basel) 101:97–103.
- Brenner E. 2014. Human body preservation—Old and new techniques. J Anat 224:316–344.
- Bryce DM, Croshaw B, Hall JE, Holland VR, Lessel B. 1978. The activity and safety of the antimicrobial agent bronopol (2-bromo-2-nitropropan-1, 3-diol). J Soc Cosmet Chem 29:3–24.
- Campbell JW, Margrave JL 1998. Preservative and embalming fluid. In: Google Patents.
- Clerc MP, Sigoure J. 2001. Composition and liquid containing this composition for the preservation of human or animal corpses. In: Google Patents.
- Da Silva RMG, Matera JM, Ribeiro AA. 2004. Preservation of cadavers for surgical technique training. Vet Surg 33:606–608.
- Davidson SS, Benjamin WH. Jr. 2006. Risk of infection and tracking of work-related infectious diseases in the funeral industry. Am J Infect Control 34:655–660.
- Dell'Amore C. 2014. Who Were the Ancient Bog Mummies? Surprising New Clues. In. Copenhagen: National Geographic.
- Desroches J, Grabs U, Grabs D. 2013. Selective ultrasound guided pectoral nerve targeting in breast augmentation: How to spare the brachial plexus cords? Clin Anat 26:49–55.
- Dissard D, Jay JC, Gilotin D, Graugnard H, Berka N. 2009. Composition for Tissue Preservation. In: Google Patents.
- Eisma R, Lamb C, Soames RW. 2013. From formalin to Thiel embalming: What changes? One anatomy department's experiences. Clin Anat 26:564–571.
- ESCO TESC. 2012. Material Safety Data Sheet. In European-Commision. 2013. Guidance document on the evaluation of efficacy of embalming products (PT22). In: Environment, editor.
- Ezugworie J, Anibeze C, Ozoemena F. 2008. Trends In The Development Of Embalming Methods. Internet J Altern Med 7.
- Fessel G, Frey K, Schweizer A, Calcagni M, Ullrich O, Snedeker JG. 2011. Suitability of Thiel embalmed tendons for biomechanical investigation. Ann Anat 193:237–241.
- Fox CH, Johnson FB, Whiting J, Roller PP. 1985. Formaldehyde fixation. J Histochem Cytochem 33:845–853.
- Frølich KW, Andersen LM, Knutsen A, Flood PR. 1984. Phenoxyethanol as a nontoxic substitute for formaldehyde in long-term preservation of human anatomical specimens for dissection and demonstration purposes. Anat Rec 208:271–278.
- Genelyn. 2010. Material Safety Data Sheet. In.
- Goyri-O'Neill J, Pais D, Freire de Andrade F, Ribeiro P, Belo A, O'Neill A, Ramos S, Neves Marques C. 2013. Improvement of the embalming perfusion method: The innovation and the results by

- light and scanning electron microscopy. Acta Med Port 26:188–194.
- Gunderman RB. 2008. Giving ourselves: The ethics of anatomical donation. Anat Sci Educ 1:217–219.
- Guo S, Schwab A, McLeod G, Corner G, Cochran S, Eisma R, Soames RW. 2012. Echogenic regional anaesthesia needles: A comparison study in Thiel cadavers. Ultrasound Med Biol 38: 702–707.
- Hammer N, Loffler S, Feja C, Sandrock M, Schmidt W, Bechmann I, Steinke H. 2012. Ethanol-glycerin fixation with thymol conservation: A potential alternative to formaldehyde and phenol embalming. Anat Sci Educ 5:225–233.
- Hayashi S, Homma H, Naito M, Oda J, Nishiyama T, Kawamoto A, Kawata S, Sato N, Fukuhara T, Taguchi H, Mashiko K, Azuhata T, Ito M, Kawai K, Suzuki T, Nishizawa Y, Araki J, Matsuno N, Shirai T, Qu N, Hatayama N, Hirai S, Fukui H, Ohseto K, Yukioka T, Itoh M. 2014. Saturated salt solution method: A useful cadaver embalming for surgical skills training. Medicine (Baltimore) 93: e196
- Holomanova A, Ivanova A, Brucknerova I, Benuska J. 2001. Andreas Vesalius-the reformer of anatomy. Bratislavske lekarske listy
- Hubbell DS, Dwornik JJ, Alway SE, Eliason R, Norenberg RE. 2002.

 Teaching gross anatomy using living tissue. Clin Anat 15:157–
- Janczyk P, Weigner J, Luebke-Becker A, Kaessmeyer S, Plendl J. 2011. Nitrite pickling salt as an alternative to formaldehyde for embalming in veterinary anatomy-A study based on histo- and microbiological analyses. Ann Anat 193:71-75.
- Jaung R, Cook P, Blyth P. 2011. A comparison of embalming fluids for use in surgical workshops. Clin Anat 24:155–161.
- Jones DG. 1997. Anatomy departments and anatomy education: Reflections and myths. Clin Anat 10:34–40.
- Jores L. 1913. Ueber eine verbesserte methode der konservierung anatomischer objekte. Munch Med Wschr 60:976.
- Kaiserling C. 1900. Ueber Konservierung und Aufstellung pathologisch-anatomischer Präparate für Schau-und Lehrsammlungen, Verhandl Deutsch Pathol Gesel 2:203–217.
- Langner RR, Jackson L. 1966. United States Patent Office. In.
- Majewski P, Pernak A, Grzymislawski M, Iwanik K, Pernak J. 2003. Ionic liquids in embalming and tissue preservation. Can traditional formalin-fixation be replaced safely?. Acta Histochem 105:135–142.
- Mason JT, O'Leary TJ. 1991. Effects of formaldehyde fixation on protein secondary structure: A calorimetric and infrared spectroscopic investigation. J Histochem Cytochem 39:225–229.
- Mayer RG 2011. Embalming: History, Theory, and Practice, 5th ed. United States: McGraw-Hill.
- McLachlan JC, Bligh J, Bradley P, Searle J. 2004. Teaching anatomy without cadavers. Med Educ 38:418–424.
- Messmer C, Kellogg RT, Zhang Y, Baiak A, Leiweke C, Marcus JR, Levin LS, Zenn MR, Erdmann D. 2010. A technique to perfuse cadavers that extends the useful life of fresh tissues: The Duke experience. Anat Sci Educ 3:191–194.
- Nawroth PP, Handley DA, Esmon CT, Stern DM. 1986. Interleukin 1 induces endothelial cell procoagulant while suppressing cellsurface anticoagulant activity. Proc Natl Acad Sci USA 83:3460– 3464.
- Neumann HG. 1974. Untersuchungen über die Anwendungsmöglichkeiten von "Merphen "als Konservierungsmittel anatomischer Präparate, Der Präparator, Bochum 20:30–35.
- Norton-old KJ, Schache AG, Barker PJ, Clark RA, Harrison SM Briggs CA. 2013. Anatomical and mechanical relationship between the proximal attachment of adductor longus and the distal rectus sheath. Clin Anat 26:522–530.
- Pabst R. 1987. Exposure to formaldehyde in anatomy: An occupational health hazard? Anat Rec 219:109–112.
- Prasad Rai B, Tang B, Eisma R, Soames RW, Wen H, Nabi G. 2012. A qualitative assessment of human cadavers embalmed by Thiel's method used in laparoscopic training for renal resection. Anat Sci Educ 5:182–186.

- Richins CA, Roberts EC, Zeilmann JA. 1963. IMPROVED FLUIDS FOR ANATOMICAL EMBALMING AND STORAGE. Anat Rec 146:241–243.
- Ryabinin IA, Razumova OS, Manucharyan LL. 2009. Biological damage to anatomical preparations. In: Proc. 17th Sci. Conf. of Students and Young Scientists with International Participation. Stavropol: Stavropol Medical Academy Press. p 563–564.
- Shoja MM, Benninger B, Agutter P, Loukas M, Tubbs RS. 2013. A historical perspective: Infection from cadaveric dissection from the 18th to 20th centuries. Clin Anat 26:154–160.
- Sigoure J, Couturier JL, Dubois JL. 2012. Compositions for Preserving the Human or Animal Body. In: Google Patents.
- Silverman ME, 1991. Andreas vesalius and de humani corporis fabrica. Clin Cardiol 14:276–279.
- Thiel W. 1992. [The preservation of the whole corpse with natural color]. Ann Anat 174:185–195.
- Titford M. 2012. Formalin Fixation. Arch Pathol Lab Med 136:137-137.
- Tolhurst DE, Hart J. 1990. Cadaver preservation and dissection. Eur J Plast Surg 13:75–78.

- Trompette P, Lemonnier M. 2009. Funeral embalming: The transformation of a medical innovation. Science Studies 22.
- Turner AJ, Mellington A, Ali F. 2005. Fresh cadaver dissection for training in plastic surgery. Br J Plast Surg 58:742–743.
- Tutsch H, Stahl G, Mörike KD, Guckes H, Arnold M. 1971. Zur Aufbewahrung anatomischer Präparierobjekte. Der Präparator, Bochum 17:85–95.
- Unger S, Blauth M, Schmoelz W. 2010. Effects of three different preservation methods on the mechanical properties of human and bovine cortical bone. Bone 47:1048–1053.
- Weiglein AH. 2002. Preservation and plastination. Clin Anat 15:445-445
- Wineski LE, English AW. 1989. Phenoxyethanol as a nontoxic preservative in the dissection laboratory. Acta Anat 136:155–158.
- Wolff KD, Kesting M, Mucke T, Rau A, Holzle F. 2008. Thiel embalming technique: A valuable method for microvascular exercise and teaching of flap raising. Microsurg 28:273–278.
- Woodburne RT, Lawrence CA. 1952. An improved embalming fluid formula. Anat Rec 114:507–514.