Improvement of the Embalming Perfusion Method: The Innovation and the Results by Light and Scanning Electron Microscopy

Aperfeiçoamento da Técnica de Embalsamamento de Cadáveres: A Inovação da Técnica e os Resultados à Luz da Microscopia Óptica e Electrónica de Varrimento

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ABSTRACT

Embalming is a chemical process that aims the preservation and sanitization of the human body indefinitely. The technique of embalming is an important tool in teaching and research in anatomy enabling the preservation of cadaveric material in good conditions (lessening any significant structural changes and maintaining the natural appearance). This article presents the results of embalmed cadavers in the course of arterial perfusion, through the use of a perfusion machine, particularly designed to this objective, and which allows the control of the embalming fluid injection process. The influence of this technique and the optimization of its parameters on the final quality of embalming were evaluated by sequential histological analysis of the cadaveric tissues using an original method of classification of samples collected from 17 deceased corpses of the Corpses Donation Office of the Department of Anatomy of Faculdade de Ciências Médicas from Universidade Nova de Lisboa, subject to the embalming technique developed in the Department. We concluded that, with this method, there is a decrease of the decomposition process at the time of embalming, which is effective at long term (over a year), requiring merely the maintenance of the body at low temperatures (4° C) and it is possible to observe that the tissue best preserved over time is muscle, showing a conservation considered optimal.

Keywords: Embalming; Dissection; Cadaver; Solutions; Microscopy, Electron, Scanning.

RESUMO

O embalsamamento é um processo químico que visa a preservação e sanitização do corpo humano por tempo indefinido. A técnica de embalsamamento é uma ferramenta importante no ensino e investigação em Anatomia viabilizando a conservação em boas condições de material cadavérico (minorando alterações estruturais significativas e mantendo a aparência natural). Este artigo reporta os resultados de embalsamamento de cadáveres obtidos por perfusão arterial, através da utilização de uma máquina de perfusão especialmente desenhada para o efeito. E que permite o controlo do processo de injecção de fluido de embalsamamento. A influência da técnica e a optimização dos seus parâmetros na qualidade final do embalsamamento foi avaliada através da análise histológica sequencial de tecidos cadavéricos e sua classificação por método original a partir de uma amostra de 17 cadáveres do Gabinete de Doação do Departamento de Anatomia da Faculdade de Ciências Médicas da Universidade Nova de Lisboa, sujeitos à técnica de embalsamamento desenvolvida no Departamento. Concluímos que, com a utilização deste método, ocorre uma diminuição do processo de decomposição no momento do embalsamamento, o qual é eficaz a longo prazo (mais de um ano), exigindo apenas a manutenção do corpo a baixas temperaturas (4° C), sendo o músculo o tecido melhor preservado, com uma classificação considerada óptima.

Palavras-chave: Embalsamamento; Dissecção; Cadáver; Perfusor; Soluções; Microscopia Óptica; Microscopia Electrónica de Varrimento.

INTRODUCTION

The high requirement level in care, technical quality, surgical and microsurgical skills, alongside with the need for a high anatomical knowledge, have recently demanded a degree of unprecedented excellence and technical mastery.[1-3] The high risk in invasive procedure performed by inexperienced hands, often, appears as a limitation in the specialist formation because of his lack of technical expertise, for example, in arthroscopy or microsurgery of the temporal bone, rarely performed.[4,5]

The current surgical training programs use a variety of instruments, including unanimated models, virtual reality, live animals and human corpses, to simulate living tissue and human anatomy and high performance of simulators for training of patient emergencies, and training in emergency teams.[1,2,6,7] Although human cadavers represent currently the most closely model to reality found in clinical practice, its cost and availability, often limited, along with the limitation of the tissues specificities severely reduce their utilization.[1,8] Table 1 summarizes some of the advantages and disadvantages of various skills training models / tools.

Embalming is a chemical process that aims to preserve and sanitize the human body indefinitely.[1] In most modern cultures, is the art of adjusting the remains to the presentation at the funeral.[9] The fear of death in modern society

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Table 1 - Types of Training instruments available skills and and their advantages, disadvantages and use situations (Adapted from Reznick R et al).

Bench Models	Cheap, portable, minimal risks.	Low acceptance by trainees. Low reliability and limited basic tasks. Not allow the simulation of an entire surgery.	Acquisition of basic skills for beginners. Improvement limited.
Live Animals	High reliability, availability, allow the practice of haemostasis and a complete surgery.	Cost, need for infrastructure ethical limitations. Use single anatomical differences.	High technical knowledge. Training situations involving haemostasis. Training dissection.
Corpses	High fidelity, currently 'only' true simulator, allows the realization of complete surgeries.	High cost, availability, possible use unique properties of tissues, risk of infection.	High technical knowledge. Dissection. Continuing medical education.
Simulation Models of Human Performance	Reusable, high reliability, data collection, interactivity.	Cost, maintenance, inactivity. Technical limitations.	Training team. Approach urgent/emergency.

have led to practices in which the corpse is viewed as looking "natural," thus denying the reality of death. It is important to acknowledge the impact of embalming, which can also carry with it problematic psychological consequences for the family.[9] From the scientific point of view, the main goal is namely to improve the body's resistance to extended periods of exposure during anatomical dissection.[1, 2, 7]

The desired properties required for successful embalming of cadavers for gross anatomy teaching include a good long-term structural preservation of organs and tissues with minimal distortion, prevention of over-hardening or appearance alteration, while maintaining flexibility of internal organs, prevention of desiccation and fungal or bacterial growth and spread within a specific cadaver and to other cadavers in the dissection room.[10]

The limitations of reproducibility of the properties of tissues, both in non-conserved body, with the potential risk of infection or accelerated decomposition, or in embalmed, conserved corpse, prevent the young surgeons from performing their learning in a safe way, without time or technical limitations, such as formalin, inappropriate for observation and approach of perforating vessels or delicate tissue dissection procedures.[3]

In 1992 was published by Walter Thiel (Graz, Austria), an embalming method with great acceptance, utilized for more than 30 years and perfected by its use in more than 977 corpses and numerous body parts. This procedure was updated in 2002.[15] It's most innovative features, compared to previous methods[2,11-13] focused on formaldehyde as a preservative, are almost no odor of the embalming fluid, ability to maintain a long-term conservation, and very minor morphological changes,[2,15] of the cadaveric material and disinfection efficacy confirmed by bacteriological tests without release to the environment of harmful substances.[2,12,17] The previous utilization of harmful substances, such as formaldehyde, raised important safety issues.[16,17] Formaldehyde levels should be measured periodically specially during the dissection in the anatomy laboratory, and local exhaust ventilation system should be installed and personal protective equipment such as safety glass and gloves should be available and be used to prevent direct skin or eye contact.[17] Therefore, prudent practices should seek to minimize formaldehyde, and other harmful substances, exposure.[12,16,18,19]

The Thiel solution fundamental basis consists of a mixture of high saline components concentration causing denaturation of proteins. The infusion is performed during three days.[2,3]

The physiological texture of tissue is maintained by the precipitation of the solution, without observation of tissue retraction or saturation. This solution has been modified, either the relative composition of its basic components as with the introduction of new compounds to allow a more adequacy to the particular tissue properties that we pretend to preserve. In general, an ideal result is characterized by conservation that lasts six months.[2,3,6]

With the Thiel's technique, the body has a pale or reddish skin.[3] Occurs detachment of superficial epithelial layers and nails. Compared with vivo, the skin remains smooth, oiled, hairless but firmer. The subcutaneous adipose tissue retains its yellow color and differentiation in adipose cells of different sizes. The shiny appearance and strength of fascias, as well as and the permeation of intermuscular spaces by vessels and neural structures is no different from living in color or texture.[3]

Currently, minimally invasive surgery and, specifically, the laparoscopic approach is the 'Gold Standard' for numerous surgeries (i.e., cholecystectomy, bariatric and anti-reflux surgery).[2] Its excellence requires a high degree of dexterity, which is directly correlated with practice and exercise. As a result, the American Society of Gastrointestinal and Endoscopic Surgeons (SAGES) and the European Association of Endoscopic Surgeons (EAES) emphasized the need

to keep up with advances in technology through validated training programs, with measurement of performance, before moving to real situations, reducing in this way the advanced laparoscopic learning curve.[1,20]

In fact, the technique of embalming is an important tool, along with other conservation techniques,[21] in teaching and research in anatomy, because it enables the conservation of cadaverous material in good condition, i.e. without significant structural changes, while maintaining the natural appearance[22,23] and its limitations may introduce an important bias in investigation when present.

The dissection of a human cadaver is an indispensable practice in general training of medical students, doctors in specialist training, research in fundamental anatomical and pathological phenomena and in the improvement of diagnosis methods and therapy,[23] assuming the, therefore, its central role in teaching undergraduate and support for postgraduate teaching, the latter with great increase in recent years.[2] A safe conservation technique, economic and accessible and that ensures the maintenance of lasting physical properties of tissues is urgently needed, both in the context of pre and post graduate. Thus, the rookie running procedures as demanding as the skin flap or performance of anesthetic procedure may, in the current context, be made by direct practice in patient.[24,25]

There are mechanisms dedicated to cadaver infusion, usually automatic or semiautomatic machines, fitted with pump units of injection to propel the solution into the embalming cadaver[26] through the arterial vasculature. However, they still lack efficiency either because there is no perfusion technology that ensures the quality of the injection, either by unknowing the exact response of the vascular system to the injection and vascular perfusion process.[23] These machines, in particular, lack of automation, control and data acquisition modern systems, allowing the optimization of the technique, so with not suitable to scientific research.

Since 2006 the Department of Anatomy, Faculty of Medical Sciences, in collaboration with the Center for Physical Research and Technology at the same University, has studied the project and development of machines to allow arterial perfusion that suppresses perfusion limitations of existing systems in the market and aims to meet the needs that embalming techniques study entails.

This article reports the study of the influence of the arterial perfusion technique and the optimization of its parameters in the final quality of the embalming through the analysis of cadaveric tissues, through the utilization of a new machine specially designed to this intent.

MATERIAL AND METHODS

Between June 2009 and May 2010 were selected 17 cadavers donated sequentially, through the Corpses Donation Office implemented in the Department of Anatomy of the FCM-UNL. The body is donated to this institution through a document written in life or a desire expressed before death, ethically and legally,[24] as predicted in Decree-Law number 274/99 from July 22, 1999, in Portuguese Republic Diary,[27] and European legal and ethical framework[28,29] for body donation for pre and posts graduate education and research, and integrated in this observational study. The embalming technique used was the cadaverous automated infusion of an embalming solution conceived by the Department of Anatomy and used for several years, using the automatic perfusion machine.

The embalming solution used is a combination of aliphatic alcohols Diethylene glycol and Monoethylene glycol (90:10) optimized in order to preserve the texture, volume, color and shape of the body and its tissues as perfect as possible, in order also to allow the disinfection and sanitization of the process.

The resultant mixture solution is a clear liquid, practically odorless, colorless and denser than water. These properties combined with adequate hygroscopic levels, good solubility in organic acids and physiologically safe. Monoethylene glycol is toxic,[30,31] the same being also true for diethylene glycol.[32,33] Diethylene glycol can lead to serious complications that may prove fatal when ingested.[32,33] This substance produces no toxic vapors at room temperature and isn't harmful at touch, unless with direct contact with the product.[32-34] The cadaver embalming procedure involves a closed circuit of embalming fluid perfusion, from the machine itself to the closed vascular system of the preserved cadaver, without submersion of the entire body, so with no direct risk added to the practice of dissection. These characteristics revealed this solution as a good choice, toxicologically comparable to glycerol,[32,33] to achieve the intended goals.

An incision was made in the right and left groin, with exposure of the femoral vessels, preparing the femoral arterial injection of bidirectional embalming solution in all 17 cadavers using cross-sectional or longitudinal in about 1 cm from the femoral artery without any previous conservation intervention than external washing with Chlorhexidine soap and the cooling during transport (in a temperature of approximately 4 - 6° C), involved in a simple cadaver plastic bag. The injection was performed using the introduction of an appropriate size cannula at the proximal femoral artery, and a lower size cannula also appropriate in the distal femoral artery, at room temperature.

The injection of the embalming solution was accomplished using a pulsed infusion at a pace between 60 to 70 pulses per minute, with the aim of mimicking normal vessels in cardiac output with recoil and variation of systolic and diastolic pressure, reducing the flow resistance and expanding the extent and scope of perfused tissues.[35]

The mean duration of cadaver vascular infusion was 30 - 45 minutes per cadaver, performed with a perfusion rate of 70 pulses / minute. The average volume of the embalming solution injected was of 7 liters per body, varying with mass and stature.

Microscopic Evaluation Morphological

Tissue biopsies were performed in all 17 corpses. The collection of tissue samples was performed in two differ-

ent chronological phases - immediate post-embalming and more than 1 month after embalming.

The choice of material to be collected for sampling was related with the importance given to the evaluation of different anatomical regions with very own characteristics of conservation and particular requirements. This option is intended to obtaining a better sense of the overall quality of embalming, with particular focus on tissues addressed by students and teachers during the dissections and future uses of the cadaverous material. In the immediate post-embalming, three fragments with dimensions of 1x1 cm were picked up, respectively: skin (the anterior thigh) muscle (muscular body anterior thigh) and buccal mucosa, this collection was repeated one month after the embalming.

In each case was also registered the cause of death and the factors of possible peri and post-embalming commitment of cadaverous structures, to avoid bias in the histological results. It was used hematoxylin eosin regular and elastin staining and an Optic Microscope for the observation of the sections of tissues.

Histological classification of methodological criteria

For the stratification of the results obtained in the histological analysis, original criteria were established based on the principles of characterization of cellular autolysis reported by M. Mckenna et al.[28]

The established criteria also integrate assessment of nuclear pyknosis and changes in cytoplasmic organelles, thereby enabling to determine the degree of deterioration/breakdown of tissue samples collected. The Table 2 lists the used criteria.

Harvests were performed with the sample placed in a jar with formaldehyde, with a single random numerical identification, due to the intersection of data and available only to the element that held all harvests, omitted to the pathologist.

After biopsies, histological blades were prepared in number of 10 for each harvest. These were stained with hematoxylin-eosin and elastin. Using this process, were observed 280 slides of samples.

A report was made for each tissue, establishing a ranking in five categories of preservation of morphological structures by microscopy (Table 3), based on the number of negative or positive levels according to the criteria of histological classification of methodological degree of cellular autolysis shown in Table 2.

RESULTS

Macroscopy

After embalming, the body revealed a pale, occasionally with *livor mortis* in regions of slope. There was no change of the nail beds, scalp or in most epithelial cells of skin layers, including changes in hairiness. During the final phase of embalming, occasionally was found the acquisition of

Table 2 - Histological Classification of methodological criteria of the degree of cellular autolysis.

Eosinophilia / cell hidropization	0 (absent) 1+ (< 25% cells) 2+ (25% < > 50%) 3+ (> 50%)
Nuclear pyknosis	0 (absent) 1+ (< 25% cells) 2+ (25% < > 50%) 3+ (> 50%)
Kariorexis	0 (absent) 1+ (< 25% cells) 2+ (25% < > 50%) 3+ (> 50%)
Disorganization of fibers	0 (absent) 1+ (focal) 2+ (in more than 3 filds 10x) 3+ (widespread)
Coagulation necrosis	0 (absent) 1+ (focal) 2+ (in more than 3 filds 10x) 3+ (widespread)

Table 3 - Classification Morphological microscopic tissue into 5 categories, according to their conservation status

A	= 2+ Excellent conservation
B	2+ e = 4 Good conservation
C	4+ e = 6+ Satisfactory conservation
D	6+ e = 8+ Conservation unsatisfactory
E	8+ Tissue in autolysis

specific and generalized cutaneous depressions and turgor - characterize in practice as 'orange peel aspect'.

Compared with the living, cadaveric skin in immediate post-embalming showed no increased resistance or detachment of skin layers or significant changes in coloration. There is, however, a slight decrease of its elasticity, objectifiable in the incision with facilitated removal of tissues. The maintenance of its features easily allowed the incision of tissues, mimicking the feel of the incision in living tissue. There was a slight increase in the swelling of tissues, with a slight increase in volume (eg diameter of the forearm) but only millimeters.

The subcutaneous tissue retained its structure and yellow color, with slightly increased consistency, and the fascia and muscular bodies maintained their resistance, intense red color and elasticity of the pre-embalming (Fig. 1). Not observed in any of embalming commitment of muscle spaces or mimicry of compartment syndrome by the injection of embalming solution. The joints remained movable without significant changes of its passive range of motion. The vessels and nerves maintained their structure and integrity, easily identifiable arteries and veins on palpation, including microns caliber vessels. The lack of color in these, due to the transparency of the embalming solution, the only difficulty was its identification and distinction.

There were no significant changes between this description and morphological observation after 1 month to 6 months of embalming. Fig. 1 illustrates the quality of the tissue preservation by the technique developed by the Department of Anatomy.

Morphological microscopy

Based on the methodological criteria of Histological Classification of the degree of cellular autolysis was possible to evaluate the overall integrity of tissues.

Total of 17 corpses analyzed.

From the analysis of Fig. 2 we can see that the skin has a high rating - Excellent maintenance – in more than half the harvest, with the rest mainly classified as good conservation at the time of embalming. These results with skin are only surpassed by the muscle (Fig. 2), the tissue with the best classification of all. This classification pattern is confirmed after more than a month of embalming.

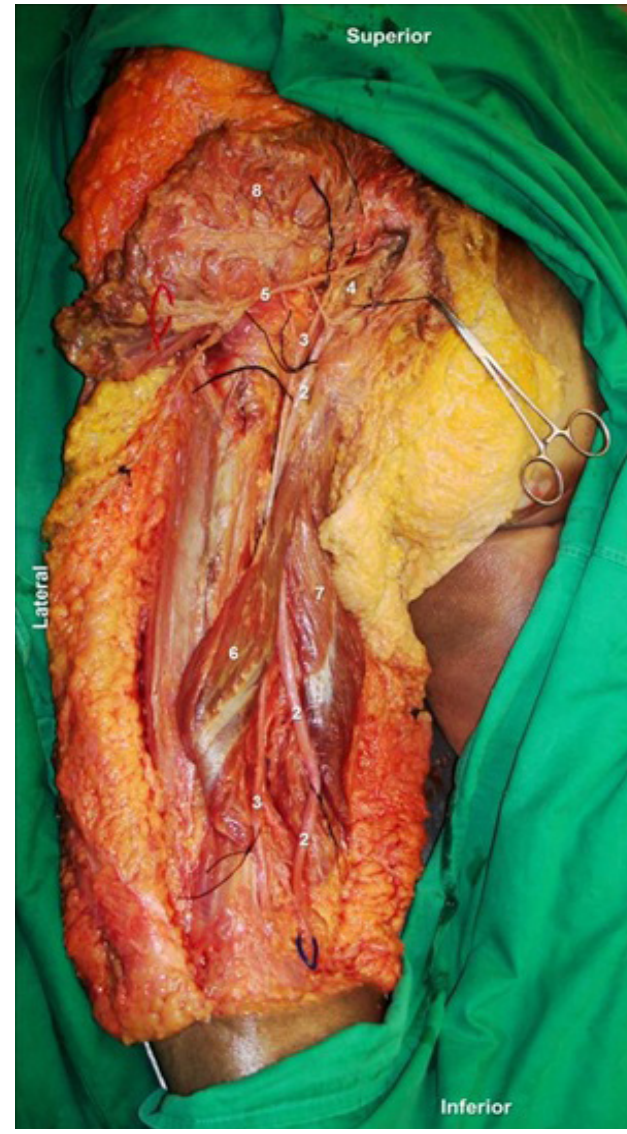

Figure 1 – Dissection of the posterior surface of the left thigh, with detail of integrity and recognition of vascular and nerve structures deep in the gluteal region in a corpse after 1 year of embalming (Images available in http://www.fcm-anatomia.com/pt/).

Comparing the long-term histologic evaluation between the 17 bodies and the 3 chosen tissues, it is possible to observe that the tissue that has the larger number of level A classification is the muscle (with a total of 6 to 8 ratings with over 1 month).

Of the three tissues collected simultaneously in each body, the buccal mucosa tissue showed the worst results in microscopic evaluation - mostly classified as B, D and E at the time of embalming and maintaining, of course, a mediocre rating in the post - 1 month embalming.

It was also performed a Scanning electron microscopy

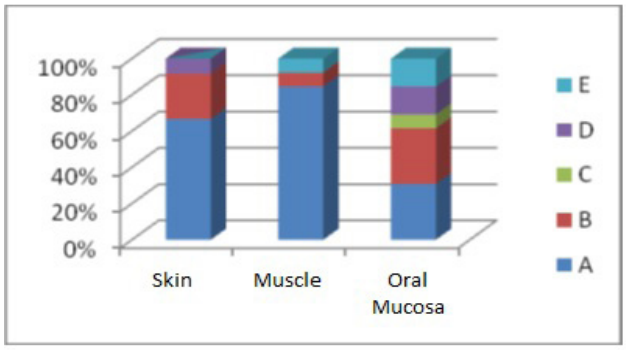

Figure 2 – Immediate post-embalming harvest.

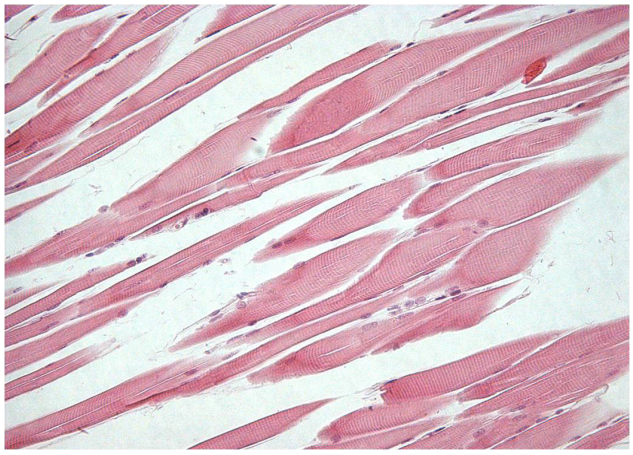

Figure 3 – Striated thigh Muscle collected after embalming (hematoxylin eosin staining – Optic Microscope, 200x).

(S.E.M.) observation of some of the cadaveric tissues, verifying structural integrity and high morphologic correlation with living tissue.

DISCUSSION

It is possible to infer that the results are generally consistent in collections held over several months, showing the same diminished/absent degree of evolution of tissue autolysis, with excellent results in cadaveric dissection, as desirable for human gross anatomy dissection.[10] It was found in almost all cadaver tissues similar resistance, mobility of joints in the various body segments and staining of structures (including organs) to the living. These properties allow unique conservation of the embalmed corpses compared to other embalming techniques, with easy recognition and individualization of nerves, arteries and veins, even at millimetric levels.

This tissue integrity is currently explored in several pregraduation courses dissection and annual regional anatomy, and in the context of post-graduated training, in surgical techniques of plastic surgery, ENT, Neurosurgery, regional anesthesia, orthopedics and urology, among others through Postgraduate Courses available through the platform of the Department of Anatomy - http://www.fcm-anatomia.com/pt/.

The prolonged maintenance of the structural integrity abolishes the limitations of previous use of cadaveric material, with the completion of current perfusion techniques for research in the Department of Anatomy of the FCM-UNL, including diafinization, angiography and Scanning Electron Microscopy in corrosion molds.

CONCLUSION

Compared with the embalming method of Thiel,[2] the embalming method developed by the Department allows a fast and effective as both macro and microscopically as shown, due to the mechanical perfusion of the body, with optimization of the procedure by its pulsed nature, allowing completion of the embalming process in less than 1 hour after the start of injection. No additional submersion of the body in conservation fluid is necessary. Most of the corpses in use with between six months and one year of embalming, present excellent texture, color and reproducibility of the techniques performed *in vivo*. This approach allows the conservation of superficial cutaneous plans, musculo-fascial and also endocavitary (i.e., abdominal, cranial). As mentioned in the method of Thiel,[2,15] the embalming solution developed and used by the Department of Anatomy – FCM-UNL does not present a direct risk to health during cadaveric material handling,[29,30] without releasing vapors into the environment and allowing a lasting disinfection of the involved tissues.

The degree of preservation obtained in skin and muscle, two of the most important tissues during the course of dissection, identification and pedagogical use of corpse pieces, explains the importance and scope of possible method adopted by the Department of Anatomy – FCM-UNL.

Comparing the long-term histologic evaluation between the bodies 17 and the 3 tissues chosen, it is possible to observe that the tissue best preserved over time is muscle (Fig. 3), showing a conservation considered optimal (less than or equal to two criteria positive autolysis cell).

The isolated discouraging results obtained for the buccal mucosa at the time of embalming, imply that the change may be pre-existing. Given the average age of the cadavers preserved by the method of embalming - average age of 60 years - and the presence of prosthetic teeth in almost all cases it is important to future investigate the possible contribution to the further deterioration of mucosa from the post-embalming immediate and its relationship to quality of embalming.

The refinement of this technique and its perfusor has allowed the realization of human cadaver embalming in the Department of Anatomy, under the dissection courses in pre and post-graduation, some already realized and others underway, where the existence of well-embalmed cadaveric material differentiates this technique in a manner fully face the other, as well as the method used so far by the scientific community.

Thus, we conclude that there is an interruption of the decomposition process at the time of embalming, which is effective at long term (over a year), requiring merely the

maintenance of the body at low temperatures, while positive (4° C), for optimization and perpetuation of results.
This technique and machine allows the preparation of cadaver embalming material in good condition, allowing pathological examinations, forensic and their long-term conservation of the cadaveric material.

The experience has shown that the preservation exceeded 5 years. We consider it important in the near future to perform tissue culture and assessment of resistance to fungal inoculation of the embalmed tissues, for additional macroscopic recognition of their conservation.

The anticipation is, in the near future, to maximize the data obtained during the embalming process, such as temperature and flow pulsatility already tested, to optimize the process and obtain a longer and lasting conservation, disrupting the tissue breakdown as early as possible.

CONFLICT OF INTERESTS

None stated.

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None stated

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