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Anatomical Basis, Histological Findings and Hemodinamics in the Modern Perfusion Model for Human Corpses

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ABSTRACT

Introduction: sSeveral modern human cadaveric fixation methods are subject to permanent evaluation. Formaldehyde is the oldest and still the most widely used method of embalming. However, the International Agency for Research on Cancer has proven its high carcinogenic potential and its use was banned, with the recommendation of research for better alternatives in the conservation of corpses. The embalming method of excellence, which preserves all features, while keeping the disinfectant properties against cadaveric decomposition was proposed by João Goyri O'Neill. Their method was considered "the most modern and efficient technique in cadaveric preservation". The aim of this present study was to analyze the quality of this original perfusion technique, at the organic level, based on central and peripheral hemodynamics. The cadaveric material was embalmed through a pulsed arterial perfusion system, connected to an automatic intermittent pump, that permits stability of the microvascular network, as well as the computerized measurement of the main perfusion parameters, such as flow and pressure. This procedure ensures good preservation of color, elasticity, texture, flexibility and fresh appearance, for several years. The morphological characteristics of the organs exhibited astonishing similarity to the living organic tissues, even several years after embalming and high freezing. Microscopic analysis demonstrated preservation of the structure of vessels, such as the aorta. Further studies on the integrity of the endocardial layer of the heart will enable to adapt the intermittent perfusion pump system to best simulate cardiac rhythm and arterial pulse, during cadaveric surgical training.
Keywords: Embalming; Gross anatomy; Perfusion; Hemodynamic; Vessel; Artery.

Introduction

The exact beginning of embalming techniques is difficult to trace with certainty because the earliest attempts to perform human body preservation may date back to the first funerary rituals. In etymological terms, the word "embalming" literally implies the impregnation of corpses with aromatic "balsams" to substitute necrotic odors, and to retard degradation of tissues (1). The funerary rituals of embalming the dead is clearly mentioned in ancient religious texts, such as the Holy Bible (Genesis 50:2; 50:26; 50:3).

We learnt from the "Ebbers papyrus" that the Egyptians proposed embalming and mummification techniques for the sacred funerary rites of pharaohs and their close family or high Priests. These embalming rituals included evisceration, addition of aromatic herbs, natron, and saline solutions, with a degree of dehydration that permitted preservation for more than three thousand years[1].

Paradoxically, we still discover traces of original mummification procedures of the Inca, Quetchuan and Chinchorro sacred funerary rites, as more recently found in inaccessible hidden shrines on the Andean mountaintops of Peru and around the Atacama Desert of Chile. These were mummified through freezing, and salt dehydration[2,3]. Pyramidal building of shrines may be correlated to the exact degree of humidity and rarified oxygen atmosphere that is more appropriate for the correct preservation of these mummified bodies. In Eastern Asia, more traces of medieval mummification were found, in China and Korea[4,5].

The funerary rituals of the emperor Alexander the Great lasted for more than three months, including a boat crossing of the Mediterranean Sea, from Greece to Memphis and Alexandria in Egypt. In preparation for the journey, the coffin of the emperor was filled with honey and herbs, in a procedure that is known as "mellification"[6]. In those days, honey was not a purified ingredient, as in common use today. It was gathered from beehives, along with the covering wax. Hence, these ancient mellification procedures may well represent the earliest sign of human body preservation through wax impregnation, as it later became originally popular in 17th century Italy[7,8].

In the Renaissance, Leonardo da Vinci developed a method of venous injection for preserving bodies, in

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anticipation of modern embalming procedures.

In the modern World, since the 18th century, several original embalming and preservation techniques were tried, for medical and academic purposes, with the scientific advancement of universities and dissection courses for medical studies and anatomy teaching.

Embalming by arterial injection as a mortuary practice began in England in the 18th century after William Harvey's discovery of the circulation of blood. William Hunter (1718-83) is "credited with being the first to report fully on arterial and cavity embalming as a way to preserve bodies for burial"[1].

In the mid-1800s, John Morgan formally established two basic principles for producing the best embalming results: 1) Injection of the embalming fluids into the largest arteries possible; and 2) Use of pressure to push the solution through the blood vessels. Quoting from the Elite Staff, 2013, "Morgan's method required that the body be opened so the heart was visible, then an 8-inch pipe was inserted into the left ventricle or aorta. The pipe was connected to yards of tubing ending in a fluid container hung above the corpse. The force of gravity acting on the liquid above the body would exert about 5 pounds of pressure, adequate to the purpose of permeating the body"[9].

Based on these principles, Thiel[10], Garrett Jr[11], Coleman & Kogan[12] and Goyri O'Neill *et al*[13] presented modern and efficient ways of preserving corpses, both for funerary purposes, as for anatomical teaching or surgical and clinical research.

For the success of any of these modern embalming methods through vascular perfusion, the hemodynamics of arterial vessels must be taken into consideration. The perfused liquid suffers variations of flow near the proximity of the vessel walls. The speed of perfusion suffers reduction near the periphery of vessels, while being enhanced near the center of the vessels. These parameters vary according to the caliber or internal diameter of the vessel. These hemodynamic aspects must be considered, when analyzing the quality of embalming methods.

Several cadaveric fixation methods are currently in use for the best preservation of human bodies. According to Healy *et al*[14], formaldehyde is the oldest and most effective and still the most widely used method of embalming. It allows preservation of the macroscopic and microscopic features of tissues. However, the International Agency for Research on Cancer[15] has proven the high carcinogenic potential of this embalming procedure and the use of this material was banned, with the recommendation of research for better alternatives in the conservation of corpses[15,16]. Nowadays the embalming method of excellence, which preserves all features, while keeping the disinfectant properties against cadaveric decomposition process, was proposed by João Goyri O'Neill *et al* (2013). Their method is referred by Brenner[17] and Balta *et al*[18] as "the most modern and efficient technique in cadaveric preservation". This latter study also mentions the necessity to further analyze the quality of cadaveric preservation through this technique, by histologic analysis at the organs level.

The aim of our present study is to analyze the quality of this original embalming technique of cadaveric perfusion, at the organic level, based on histologic analysis, and central and peripheral hemodynamics.

Material and Methods

All the material here presented results from fully legalized donations of human corpses. All were embalmed by the Goyri O'Neill[13] technique that permits good preservation of flexibility and fresh appearance, for several years.

The corpses were embalmed with a system of arterial propulsive perfusion, connected to an original automatic intermittent pump (Figure 1). This permits control of the embalming fluids perfusion, with a frequency of 1 Hertz, to allow stability of the microvascular network, as well as the computerized

Figure 1. Perfusion system with computer program.

measurement of the main vascular perfusion parameters, such as flow and pressure. This system allows dosage of a constant flow of embalming liquid, at the rhythm of 60-70 pulses per minute, in simulation of the cardiac rhythm. The pressure in current use varies from 1 to 7 bar, to allow a nearly physiological embalming flow with the adequate perfusion that impregnates even the smallest capillary vessels, up to the most peripheric regions of the body.

To improve the quality of these perfusion parameters, we must consider patterns of the cadaveric characteristics, such as age, gender, weight, and height (Body Mass Index), previous pathologies, "causa mortis", and the duration between the time of death and the embalming perfusion. Furthermore, the hemodynamic properties of the embalming fluid must also be taken into consideration, for best results in terms of the uniformity of the whole-body distribution. These include the viscosity, temperature, osmotic quality, and pH of the perfused fluid, regarding the liquid friction strain against the vessel wall, in direct relation to the characteristics of the vessels, and according to their inner caliber[19].

The current embalming technique is performed after regional dissection of the origin of the femoral artery (Figure 2) to allow introduction of two catheters, one in ascending direction and the other descending, to connect both to the intermittent automatic pump that reproduces the heart rate, blood flow and body temperature, thus ensuring perfusion of every vessel in the corpse. The duration of a whole cadaver perfusion varies according to the cadaveric characteristics and BMI. It may take between 30 to 70 minutes to perfuse an average of 10 litres of product.

The embalming fluid corresponds to a mixture of aliphatic compounds, including Diethylene glycol and Ethylene glycol in a proportion of 90:10. These proportions have been tested as the best to allow the similarity with preservation of the characteristics of the living human body, including flexibility, elasticity, and odour, while keeping disinfectant quality.

The viscosity of the embalming fluid was considered in relation to the temperature of 37°C at which the liquid is perfused. We considered an average viscosity of 26.7 lbm, which permits the ideal perfusion of all the arterial and capillary networks in the body.

The embalmed cadavers are then kept in high freezing chambers, at a temperature of -20°C to -30°C, for over a year, to ensure the permanent quantity of corpses in stock at the Anatomy Department. They are then transferred to cold chambers after defrosting for anatomical works, during which they can be kept in refrigeration chambers, at a temperature of 4-6°C.

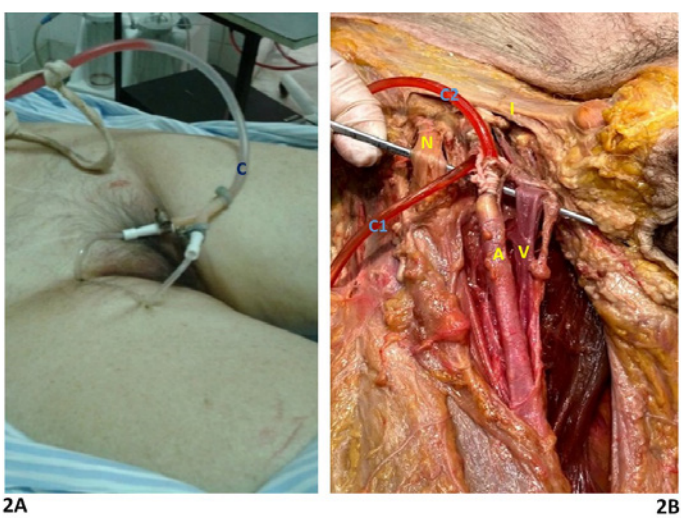

Figure 2. Injection of the embalming fluid through the right femoral catheter. 2a-Technical aspects of the femoral artery catheterization. "c" - Embalming catheter; 2b – Successful undergraduate students' dissection of the femoral triangle, around the point of introduction of the embalming catheter. "c1"- Embalming catheter, introduced in ascending way; "c2"- Embalming catheter, in descending way; "I"- Inguinal chord; "N"- Femoral Nerve: "A"- Femoral artery; "V"- Femoral vein.

Dissection and histology

For this present study, we removed five aortic arches from corpses that had been embalmed for one to three years. The aortic arches were obtained after careful dissection of five rib cages. Histological preparations from thin slices of the aortic wall, after staining with hematoxylin and eosin (HE) and Veroeff techniques, provide good analysis of the histologic features of the aortic arches' walls, according to different embalming techniques. The histologic preparations were analysed with light microscopy and photography.

The histologic preparations of the five aortic arches collected after embalming perfusion were compared with similar material, prepared from aortic arches collected from fresh non-embalmed cadavers, and used as control for the present study (Figures 5a; 6a).

Results

Macroscopic results after gross dissection

All the corpses that we analyzed had good preservation of the color, elasticity, and texture of the skin (Figure 3), as confirmed by bibliographic references[13,20,21]. Regarding the preservation of the organs, their morphological characteristics are well preserved, ensuring good similarity to the organic tissues of the living individual.

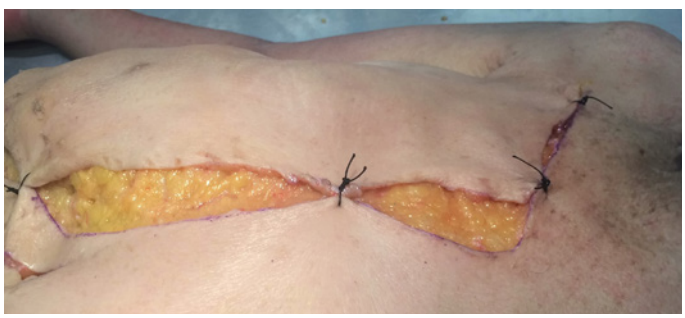

Figure 3. Aspects of freshness of the skin and subdermal adipose tissue in a cadaveric body prepared by the Goyri-O'Neill technique. These gross dissection specimens are odorless, and our students take such pleasure in dissecting that they usually request for extra hours of work, largely exceeding the scheduled time for their classes.

We acknowledge the works of the undergraduate medical students at the Nova Medical School, for their dissection works (Fig.2B) that fully demonstrate the adequate end-results of this embalming technique in macroscopic terms for gross dissection, and also for post-graduate surgical training and research.

The photographic proof presented in Figure 3, clearly demonstrates the good quality of elasticity and freshness of the subdermal layer of adipose tissue, on gross dissection of a cadaver, embalmed with this technique. In fact, in macroscopic terms, this is the best demonstration of the success of this embalming technique to impregnate peripheric capillary networks, so rich in these subdermal layers of the human body.

Dissection and removal of the aortic arches demonstrated the good quality of preservation of their macroscopic and microscopic characteristics, even several years after embalming and high freezing, regardless of the cause of death (Figures 4, 5 and 6).

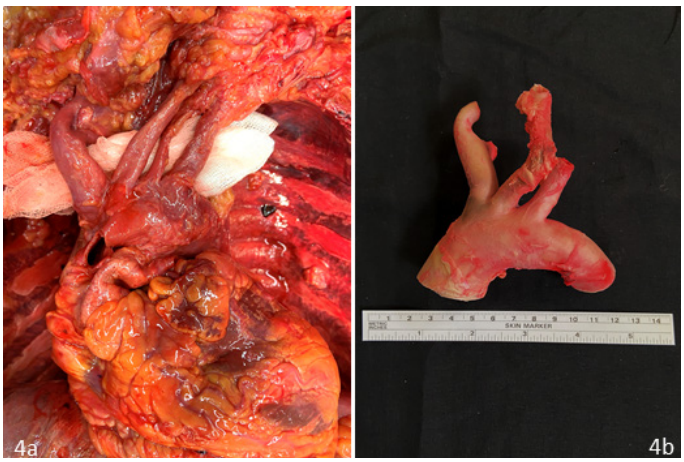

Figure 4. 4a and 4b: dissection of the aortic arch.

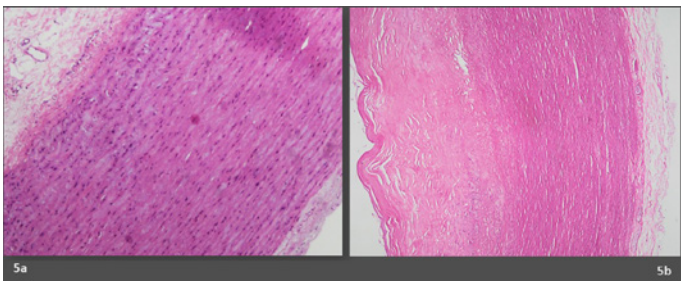

Figure 5. 5a: histologic slide with HE staining of the aortic arch obtained after dissection of an embalmed cadaver. 5b: histologic slide with HE staining of the aortic arch obtained from fresh, non-embalmed cadaver.

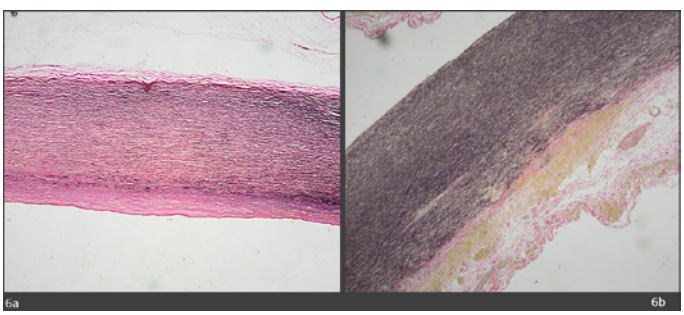

Figure 6. 6a: histologic slide with Veroeff staining of the aortic arch obtained after dissection of an embalmed cadaver. 6b: histologic slide with Veroeff staining of the aortic arch obtained from fresh, non-embalmed cadaver.

Histological Analysis

The analysis of Figures 5b and 6b, obtained from aortic samples prepared with HE and Verhoeff staining for optical microscopy, clearly demonstrate good preservation of all the histologic layers of the arterial wall. The endothelial lining exhibited good integrity, and no signs of disruption, after the passage of the embalming fluid.

Furthermore, we performed a comparative study with samples collected from fresh, non-embalmed cadavers, prepared by the same techniques, in the same pathology lab, to verify our first quick impression that the histologic preparations from embalmed corpses exhibit good definition and separation of layers, both in fresh material, as in the samples collected from the embalmed cadaveric material, kept in high freezing chambers, for several months.

In any case, the permanent monitoring of the times, temperature, and humidity of the working environment in the embalming room and freezing chambers, is seemingly important. (Figures 5a and 5b; 6a and 6b).

Hemodynamic aspects

The observations we gathered from macroscopic and microscopic analysis of samples were performed after careful monitoring of several hemodynamic parameters, that were calculated to reach the best quality of tissular impregnation by the embalming fluids. The liquid flow and pressure of the propulsive embalming pump were calculated to reduce the friction strain against the inner layers of the vessels. In search for the best embalming parameters, we also monitored the embalming fluid characteristics, such as temperature or pH, and the working environment temperature and humidity, We learnt from our historical notes, on the importance of these parameters for the best end-result quality of the embalming procedures.

Discussion

In face of the present results, one can easily infer that the adequate monitoring and control of the hemodynamic aspects of the propulsive embalming pump and the quality of the embalming fluid allow the preservation and integrity of the cadaveric vessel walls for anatomical research and surgical training.

In his works, Balta *et al*[18], comments the technique that we present here, with mention to the embalming fluid that preserves human corpses for periods of over one year, as originally analysed by Goyri O'Neill *et al*[13]. Pfeil *et al*[22] further completes this previous analysis with the comment that this technique does not fulfil the requisites for the ideal embalming.

We proceeded our own researches on the Goyri O'Neill technique with the histologic analysis of aortic arches collected from embalmed corpses that had been preserved for over 3 years in high freezing chambers, thus demonstrating that even after such

long conservation times, the endothelial lining, the main histologic characteristics and the best, near to "*in vivo*" tissular quality are kept, when careful hemodynamic parameters control is ensured during the embalming procedures. These results are well demonstrated in Figs.5-6.

Much of the most recent research led us to the undeniable fact that human anatomy teaching is more effective when the subject is taught with the practical fundament of anatomical dissection. Nevertheless, such practice has slowly been suffering gradual decline in worldwide medical schools, in consequence of a multitude of technical factors, which include the toxicity of embalming liquids such as formaldehyde, which is still the most widely used embalming fluid, despite of its confirmed toxicity and carcinogenic effects[15,23-27].

Furthermore, the use of formaldehyde results in tissular dehydration, with consequent rigidity and loss of colour of the anatomical specimens. It enhances blood clotting and capillary retraction, thus limiting surgical plans and hampering the recognition of smaller structures[17,27,28].

In perfect contrast to these previous limitations to the use of formaldehyde, the present embalming technique with the addition of an original propulsive pump allows technicians, researchers, students, and demonstrators to work under much more comfortable and secure ambiance, both for the embalming procedures, as for human anatomy teaching, or for surgical training and research[20,29]. The embalming fluid is intermittently instilled through the arterial system with full impregnation of the peripheral capillary networks, thus preserving the elasticity and near-living appearance of the human cadaveric material.

This lesser toxicity combined with the preservation of colour, flexibility and "near-living" texture of cadaveric specimens is essential for successful end-results in surgical training postgraduation courses. Thus, this modern technique comes in perfect contrast to most of the hampering negative qualities detected in previous embalming techniques, including their high toxicologic/carcinogenic effects[27,30,31].

Cadaveric embalming perfusion methods have been subject to several modifications along the years, according to the evolution of the embalming fluids in use. Originally and for quite some time, a method of gravidity in closed circuit was used. For this purpose, a recipient containing the embalming fluid is elevated above the cadaver, for instillation through the carotid or femoral arteries[22,28,32].

Nesbitt *et al*[29] developed the project of a new model of pulsatile circuit for human cadavers to use in surgical training after high freezing. They applied hemodynamic patterns to ensure the perfusion fluids to reach the most peripheric regions of the body. The authors applied the perfusion of a saline solution to remove possible intravascular clotting. The following perfusion fluid corresponded to glycerol in saline perfusion, thus reproducing the blood viscosity. These techniques are applied under ambient temperature to avoid quicker degradation of tissues, in case of fluid warming.

In future publications, our group of researchers will develop and analyse further implications of this technique in view of the hemodynamic aspects involved.

Conclusions and Perspectives

Previous studies have demonstrated that the Goyri O'Neill's Technique[13] fulfilled the request proposals of Balta *et al*[18] in terms of the quality of preservation of human corpses.

Our continued research intends to seek for new information on the preservation of human organs with this original embalming technique. It is important to promote more comfortable working environments both for undergraduates' dissection classes, as for surgical training, and for anatomical researchers or for research in developing and improving new surgical techniques. This embalming fluid is odorless, as no other. This allows closer proximity from the performers during surgical procedures. It provides corpses with the elasticity and appearance and many characteristics that are in close similarity to those of living human bodies. In macroscopical terms, J. Goyri O'Neill has already widely demonstrated the overall success of these embalming techniques that preserve the elasticity and "near-living" appearance of human cadaveric material, for anatomical education purposes and above all for surgical training courses[20,21]. The Anatomy Department of the Nova Medical School is well equipped to receive several "hands-on" postgraduation courses, and Surgical Training courses, on yearly basis, with persistent success.

Furthermore, microscopic analysis also demonstrated good preservation of the structure of vessels, such as the aorta. Our future studies will analyze other vessel walls, their structures, microscopic features, geometry, and hemodynamics. To ensure the credible functional link with the hemodynamic parameters, we will further analyze through the same methodology, histological samples of more peripheral arteries, such as the limb arteries, and some samples from microvascular networks of vascular organs, such as from the liver or the spleen. We intend to compare these results with the structure and histological features of non-embalmed cadaveric material.

Our present first results indicate the interest for further monitoring of the several parameters and results from this embalming technique, to ensure the formal guarantee that it is in fact the best alternative to substitute the use of formaldehyde

from international anatomical dissection rooms. We intend to apply these technical innovations in Brazil, to monitor the results in different atmospheric ambiance of embalming and dissection works.

Furthermore, we will verify if this integrity of the endothelial layer of larger vessels also applies to the smaller vessels and microvascular networks of organs, through Scanning Electron Microscopy, as currently in use at the Nova Medical School, to demonstrate the vascular architectural arrangement of organic vessels.

In fact, as soon as these parameters are verified, the next step to further ameliorate this embalming technique will be to adapt the hemodynamic parameters of the computerized intermittent pulse pump, to enable circulating pulse that ensures human cadaveric peripheral arterial pulse and cardiac rhythm to best simulate the human living conditions during surgical training courses and research.

Acknowledgements

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