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Original article

Viability and characterization trial of a novel method as an alternative to formaldehyde and Walter-Thiel cadaveric preservation for medical education and surgical simulation

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ABSTRACT

Introduction: Despite its toxic and carcinogenic nature, formaldehyde is a widely used reactant for specimen preservation. With the need of specimens for both anatomical and surgical training, alternative preservation solutions (PS) have been proposed, however, their use is limited due to high costs and complexity. Hence, a new formaldehyde-free solution (FFS) is evaluated as a potential alternative for anatomical and surgical training.

Methods: Qualitative and Quantitative data were acquired. Specimens preserved using three different methods were selected. Flexibility was measured by joints goniometry and pneumoperitoneum pressures were evaluated followed by an exploratory laparoscopy. Undergraduate student's perceptions on cadavers preserved with different PS were obtained using surveys and focus groups.

Results: The main reason why cadaveric specimens were considered as useful tools was the perceived interaction with real tissues and the 'practical' concept of getting in touch with what students would be facing in the future as physicians, what we call ''hands on'' activities. FFS treated specimens showed better joint-movement ranges in comparison to other methods and pneumoperitoneum was acquired after 5 mmHg CO_2 pressure. Students appreciated working with corpses regardless the technique used, however FFS specimens were defined as less uncomfortable, while presenting no sensory discomfort.

Conclusions: Even though alternative PS are effective, high costs and complexity restrict their usage. Cadavers preserved with FFS had similar range of movements compared with

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Thiel. Students preferred to work with FFS rather than FF due to flexibility, color, and no sensorial hassles. Thus, we propose FFS as viable alternative to traditional PS.

Viabilidad y prueba de caracterización de un nuevo método como alternativa al formaldehído y la solución de Walther-Thiel para la preservación de cadáveres para educación médica y simulación quirúrgica

Palabras clave:
Simulación quirúrgica
Anatomía
Entrenamiento
Simulación
Educación
Agentes fijadores
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RESUMEN

Introducción: A pesar de su naturaleza carcinogénica y tóxica, el formaldehido continúa siendo utilizado para preservar especímenes. Debido a la necesidad de especímenes para entrenamiento anatómico y quirúrgico, se han propuesto soluciones preservadoras (SP) alternativas; sin embargo, su uso es limitado debido a los altos costos y a su complejidad. En consecuencia, se evalúa una nueva solución libre de formol (FFS) como una alternativa para el uso en entrenamiento anatómico y quirúrgico.

Métodos: Se obtuvieron datos cualitativos y cuantitativos. Se seleccionaron especímenes preservados utilizando diferentes métodos y se analizaron biopsias de cada uno. Se midió la flexibilidad mediante goniometría, y se evaluó la presión del neumoperitoneo. Utilizando encuestas y grupos focales se obtuvo la percepción de estudiantes de pregrado respecto a cadáveres preservados con diferentes soluciones.

Resultados: Los principales motivos por los que los estudiantes refirieron percibir los cadáveres como herramientas útiles fueron poder interactuar con tejidos reales y el concepto de «practicidad» generado por actividades percibidas como similares a la práctica como médicos profesionales. Los especímenes tratados con FFS demostraron mejor movimiento articular en comparación con otras soluciones, además de lograr neumoperitoneo con 5 mmHg de CO_2. Los estudiantes refirieron sentir menos molestias sensoriales al utilizar cadáveres preservados con FFS.

Conclusiones: Aunque otras SP son efectivas, los altos costos y la complejidad restringen su uso. Cadáveres preservados con FFS presentan arcos de movimiento similares a Thiel. Los estudiantes prefirieron trabajar con FFS en vez de FF, debido a la flexibilidad, el color y la ausencia de molestias sensoriales. Proponemos FFS como una alternativa viable a las SP tradicionales.

Background

Gross anatomy as a fundamental medical education subject provides students a first approach to the human body.[1] Through it, students acquire concepts that are essential for comprehending various pathologies as well as clinical problems.[2] Therefore, Anatomy should be taught from a clinically significant context[3] so that students can understand its relevance in their future clinical practice.[4] Surgical simulation as an additional clinically relevant context can hence both medical student's related anatomical knowledge and increase resident's exposure to procedures while improving surgical skills in a safe environment.[5]

Multiple tools for Anatomy education have been developed through the years, giving rise to two educational trends, both of which try to answer an already described crisis in its teaching[6]: The modernist approach focuses on the use of technology as a tool, promoting virtual interaction, more efficient time usage[7] and a more realistic contact with clinical routine,[8] and the classical approach advocating traditional tools such as cadaveric dissection, considered key for the personal and professional development of future practitioners. Dissection is also considered a high impact activity both emotionally and psychologically; even regarded by some as an essential tool in medical practice[9]; it is promoted by several anatomists and educational institutions[10] since it might improve the acquisition of anatomical knowledge. From 165 countries with medical schools, 71 have available information concerning corpse usage, and 68 currently use cadavers for anatomy teaching purposes.[11] Similarly surgical simulation as training for residents falls under these educational currents, virtual reality tools and simulation on human cadavers or live porcine models have both been regularly employed and studied as means to better train surgical skills in surgery specialties.[12,13]

Corpses for education are embalmed following a variety of chemical processes to preserve and sterilize them. For Anatomy teaching as a basic science, the most commonly used preservation compound reported is formaldehyde

fixation (FF), used in up to 87% of medical schools.[14] FF has several advantages such as proper tissue fixation, conservation, and an affordable price. However, it has an important drawback: toxicity. The International Agency for Research on Cancer (IARC) classifies it as carcinogenic; nasopharyngeal cancer was highly associated with formaldehyde exposure according to the 2005 IARC Monograph in which a group of experts critically reviewed pertinent scientific literature about FF potential to cause cancer in humans.[15] Significant risk is found in occupational scenarios, where excessive levels saturate metabolic pathways and may become genotoxic.[16] High formaldehyde concentrations are also found in the academic scenario, mainly dissection rooms[17] and surgical simulation scenarios. While several attempts have been made to minimize the use of this substance, particularly in the European Union,[18] no country has banned its usage to date. Moreover, the results obtained with FF to supply the need of cadavers in advanced medical training as simulation does not allow a proper environment to learn. FF exhibits poor tissue appearance and fresh tissue characteristics loss like flexibility and color, while limiting surgical simulations due to inability to generate pneumoperitoneum.[19] A new necessity in specimen preservation appeared then, in which tissue characteristics became of the outmost importance.

To overcome these problems, new techniques have been developed such as the use of salt saturated solutions[20] and Walter Thiel, which represents an effective preservation method with minimal toxic chemicals concentrations, it ''allows a longer preservation, retaining fresh specimen qualities (color, texture, plasticity and flexibility)''.[21] This preservation solution (PS) is comprised of two solutions, A and B, the first one contains boric acid, ethylene glycol, ammonium nitrate, potassium nitrate, and hot water, while the second one contains ethylene glycol, and 4-chloro-3-methylphenol. Specimens are first injected with a mixture of solution A and B, formaldehyde, and sodium sulfate, and later immersed in a solution containing ethylene glycol, trace amounts of formaldehyde, solution B, boric acid, ammonium nitrate, potassium nitrate, sodium sulfate and hot water.[22] Thiel's solution has disadvantages such as high costs, complexity and the need of training for results to be propper.[23] Therefore, we propose a novel formaldehyde-free solution (FFS) that produces softer, more hydrated corpses with a fresher color in comparison to FF as a suitable alternative for anatomy education and surgical simulation. The proposed solution is a proprietary recipe comprised of Sodium Chloride (NaCl), Aminometanamide (CH_4N_2O), Glycerine, Ethylic Alcohol, Benzalkonium Chloride, and Citronella essential oil. Specimens are initially cannulated through the femoral arteries and veins, drained, and cleaned after which 50 l of FFS are circulated and impregnated in the corpse through the femoral vasculature. Specimens preserved with this technique are stored between 12 °C and 15 °C and covered with towels saturated with the solution which are replaced weekly. Specimens have been preserved satisfactorily for up to 3 years. Immersion in the solution for a week every 6 months is required to maintain the specimens. FFS can be safely and effectively stored between 12 °C and 15 °C avoiding exposure to direct sunlight without the need for other special considerations.

To date specimens preserved using FFS have allowed for a variety of surgical simulation procedures, including thoracoscopy and knee arthroscopy, no previous attempts at pneumoperitoneum have been carried out, therefore our aim is to describe the results when applying this solution through joint flexibility evaluation, viability for laparoscopic surgical simulation and qualitative characteristics based on student's perceptions.

Materials and method

For anatomy education viability, we conducted a single-blind non-randomized cross-sectional study comparing three cadavers preserved with three different methods: Formaldehyde fixation, FFS, and a previously FF specimen treated with FFS (FFS-FF) (see Appendix 1). After IRB approval, second year medical students taking the anatomy course at the time, with no surgical training, with previous experience with FF cadavers, who voluntarily accepted to participate in the study and signed an informed consent were asked to manipulate them as part of a scheduled lesson without knowing which preservation method was used for each specimen. Participants were surveyed ($n = 29$) after the intervention and a focus group ($n = 7$) was created with voluntary participants from the initial 29 students, to identify student's perceptions and allow for data triangulation.

To characterize resulting specimens, three evaluations were performed: Joint goniometry, histological analysis, and Pneumoperitoneum feasibility.

Goniometry measures were taken using the Washington State Department of social and health services Range of Joint Motion Evaluation Chart (DSHS 13-585A (REV. 03/2014)) [Appendix 4], focusing in three joints (hip, elbow, and wrist) for eight movements (flexion, extension, abduction and adduction) from the three specimens for comparison. A member of our team was subjected to these measurements as control.

Biopsies of three different tissues for each specimen (skin, rectus abdominis muscle and small bowel) were taken. These were processed for examination using conventional hematoxylin-eosin stain to assess histological changes caused by the different preservation techniques.

Finally, pneumoperitoneum feasibility trial was conducted with a Veress needle in each corpse measuring pressure to determine which of the methods allow a proper environment for laparoscopic surgery simulation. Specimens were placed in supine decubitus and a Veress needle connected to a CO2 system was introduced following common surgical protocols. An intra-umbilical skin incision was performed with the blade incising from the center of umbilicus caudally. The abdominal wall was then splinted with the non-dominant hand while the Veress needle was inserted with the dominant hand using continuous pressure until the sensation of loss of resistance appeared. Then, CO_2 increasing pressure starting at 1 mmHg was applied through a DRUVA aneroid gas secured system valve. External anatomical modifications until adequate pneumoperitoneum (abdominal cavity insufflation) were recorded or until a top 30 mmHg CO_2 pressure if no insufflation was acquired, gas loss was evaluated, and data gathered.

Specimen eligibility

Eligibility was based on the preservation technique performed. Eligible specimens must have been preserved with only one technique and should not have been used for any additional procedure (i.e. vessels repletion, visceral insufflation, muscles/vessels stain). Three corpses were chosen: one preserved with a 4% formaldehyde solution, one preserved with the FFS and one that had undergone 10% formaldehyde fixation and was later embalmed with FFS (see Appendix 1 for detailed descriptions of the processes). All corpses have had been fixated and treated at least twelve months prior to the study.

Intervention

Participants were asked to interact with the three corpses preserved in different solutions and asked to answer a survey about their experience while manipulating each cadaver. The survey consisted of five items scored using Linkert scales: (1) general satisfaction with each solution – scored from 1 to 10, 1 being extremely unsatisfactory and 10 being extremely satisfactory –, (2) sensory hassles, which evaluated statements associated with hassles related to sight, touch and smell, as well as perception on these sensory experiences with the tissues, they were scored from 1 to 4, 1 being very little and 4 being a lot, (3) preferences on using the solutions, in which participants were asked to organize which tissue they would prefer to work with from least preferable (1) to most preferable (3), (4) similarity to fresh tissue in which participants were asked to organize from least similar to fresh tissue (1) to most similar to fresh tissue (3) and (5) perception of usefulness as an educational tool, where they scored each tissue from not useful to 5 very useful (see appendix 2). Each specimen was identified with a letter for the students to qualify to ensure blindness: Cadaver A was FFS-FF treated, Cadaver B was FFS fixated and Cadaver C was FF.

After the survey, focus groups were created, recorded, and subsequently transcribed for analysis. The questions included in the survey and the focus groups are presented in Appendices 2 and 3 respectively. Survey and focus group questions and structure were validated by expert third parties in education and qualitative research methods ($n = 2$).

Statistical analysis

Univariate and bivariate analysis was performed for survey results; averages were calculated for each point and associations between answers and type of cadaver (i.e. method used for preservation) were evaluated by means of p values. A one-way ANOVA was performed for evaluating differences between corpses in regards goniometry results. Data was analyzed and graphed with Graph Pad Prism 8.0 for MacOS.

Results

Survey results

General satisfaction with each solution

All participants considered both FFS and FFS-FF as highly satisfying (6–10), while only 17.24% considered FF highly satisfying, 37.93% considered it to be unsatisfying (1–3), while 44.82% said it was somewhat satisfying (4–6).

Sensory hassles

Perceptions on unpleasant stimulus usually associated to specimen fixative reactants[24] are summarized in Fig. 1. And specimen qualified aspects in Fig. 2. All participants scored FF

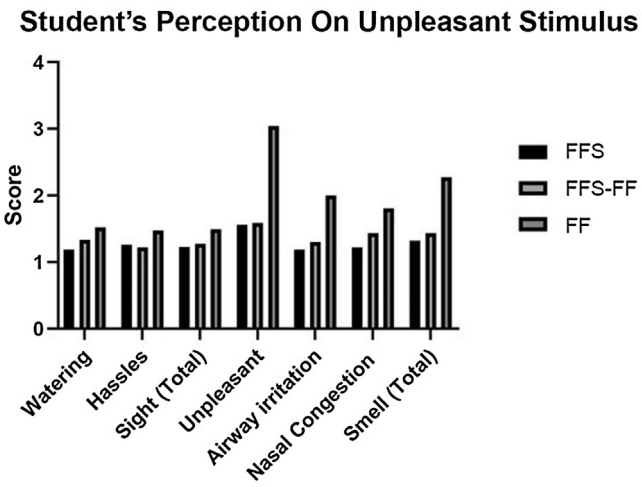

Fig. 1 – Students' sensorial experiences focused on unpleasant stimuli with each specimen preservation method. Eye watering, Hassles regarding ocular irritation, Sight (Total) includes both watering and hassles, unpleasantness when interacting with the specimen, Nasal Congestion, and pungent smell were evaluated. Calculated averages are presented. 0: I did not feel it 5: I felt it a lot (Annex 1).

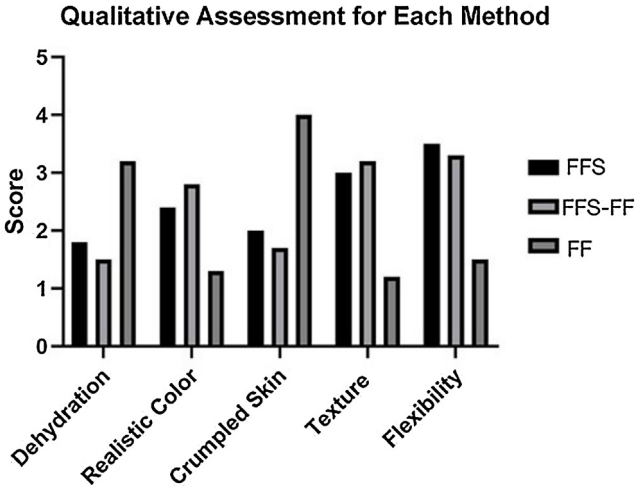

Fig. 2 – Students' qualitative assessment of each tissue. Dehydrated appearance of the tissues, Subjective evaluation of color compared to fresh tissue, Subjective evaluation of crumpled appearance of the tissues, Texture and suppleness of the tissue, and Flexibility of the specimens were scored by participants using Likert scales. Calculated averages are presented. 0: It did not have that characteristic – 5: It had that characteristic.

higher than FF-FFS AND FFS when asked about unpleasant stimuli, whilst rating FF-FFS and FFS similarly (Fig. 1). Students found FF to be more dehydrated, less realistic in color, presented crumpled skin, less elastic texture and less flexible than both FF-FFS and FFS, between FFS and FF-FFS students found FFS to be more flexible than FF-FFS, while finding FF-FFS to be more hydrated, realistic in color, elastic in texture, and less crumpled.

Preference

Regarding preference when using the tissues most students placed as most preferable FFS (55.17%), followed by FF-FFS (37.93%) and finally FF (10.34%), as second most preferable FF-FFS was the most common (51.72%) when compared with FFS and FF (37.93% and 6.90% respectively), while FF was the most common least preferred tissue (82.76%), while only 10.34% placed FF-FFS as least preferable, and 6.90% had the same preference on FFS.

Similarity to fresh tissue

Most participants considered FFS to be the preserved tissue most similar to fresh tissue (62.07%) when compared to 24.14% who thought FF-FFS was the most similar, and 6.90% who perceived FF as the most similar. 65.52% of participants considered FF-FFS to be the second most similar when compared to FFS and FF (31.03% and 6.90% respectively), while most students considered FF to be the least similar to fresh tissue (86.21%) when compared to FF-FFS and FFS (10.34% and 6.90% respectively).

Usefulness as an educational tool

FF-FFS and FFS both were considered by participants as useful learning tools, both scored 4 points on average on the Linkert scale. According to participants perception FF was not useful with an average score of 2 on the Linkert scale.

One-way ANOVA were calculated for each item in the questionnaire to find if there was any difference between the tissues evaluated. The p-value ($\alpha = 0.05$) for each comparison is shown in Table 1.

There were no statistically significant differences between FFS-FF and FFS. Nevertheless, we found statistically significant differences between FFS-FF and FF, and between FFS-FF and FF.

It is important to note that 4 of the students participating in the study have never been able to interact with a human body until the experience during the research and no significant differences between students with and without previous experience with corpses were found when comparing their working preference with preservation techniques ($p = 0.61$).

Table 1 – p-Values of one-way ANOVA calculated for respective items evaluated in the questionnaire for the three different solutions.

Questionnaire item	FFS Vs FFS-FF (p-value)	FFS Vs FF (p-value)	FFS Vs FF (p-value)
General satisfaction	0.8049	<0.0001	<0.0001
Working preference	0.4427	<0.0001	<0.0001
Useful	0.9883	<0.0001	<0.001
Sensory hassles	0.0681	0.0192	0.0367
Perception	0.9585	0.9326	0.9584

Focus groups. Students liked working with corpses regardless of their preservation technique. They reported it felt more realistic than other learning tools (books, software, images, and atlas) and perceived it helped them to enhance their knowledge.

All specimens are definitely considered as useful tools, since these let them compare and contrast between what books and models show and what a real human may look like, even some comments regarding the ethics of their use on medical praxis raised, showing the importance of students interaction with human specimens during education. They also stated that the preservation technique is important, since their experience may be affected if corpses are not in the best conditions. Students preferred to work with the specimens preserved with FFS and FFS-FF which, according to what they said, had minimal drawbacks, were easier to manipulate, more flexible, hydrated while muscles and viscera were easily distinguishable from each other. However, they did not like working with the FF specimen since it had a strong odor, it irritated their eyes and its stiffness made it difficult to handle.

The main reason why specimens were considered as useful tools was the perceived interaction with real tissues and the 'practical' concept of getting in touch with what students would be facing in the future as physicians, what we call ''hands on'' activities.[25]

Although there are several tools to explore the human body in an educational context, all students believe that the use of specimen is fundamental for their learning. Finally, students expressed that comfort and similarity with fresh/live tissues were key factors in rating one technique over another and the absence of ocular and/or airways irritation played an important role on their decision, which, as already stated, may have caused bias during the survey.

Histological results. All biopsies were stained with Hematoxylin–Eosin for inspection, both FFS and FFS-FF recovered tissues preserved basic structure, however borders between the different layers of the skin and small bowel, and hydrologic details of muscular tissue (such as strictures and cytoplasm delimitation) could not be identified. Nuclei were absent in almost every cell.

Formolized tissues however, maintained their morphology intact and well preserved.

Joint goniometry. Specimens preserved without formaldehyde at measurement had better joint movements than formaldehyde preserved specimen as shown in Fig. 3.

Pneumoperitoneum trial. As already mentioned, during experiment al corpses were kept in supine decubitus. No additional positions were tested. CO_2 pressures were recorded and are in Table 2.

After pneumoperitoneum was acquired, an umbilical trocar was inserted, and an exploratory laparoscopy pursued. Although pneumoperitoneum was acquired for both FFS-FF and FFS preserved specimens, only adequate movement arcs and intraperitoneal visualization were acquired with FFS

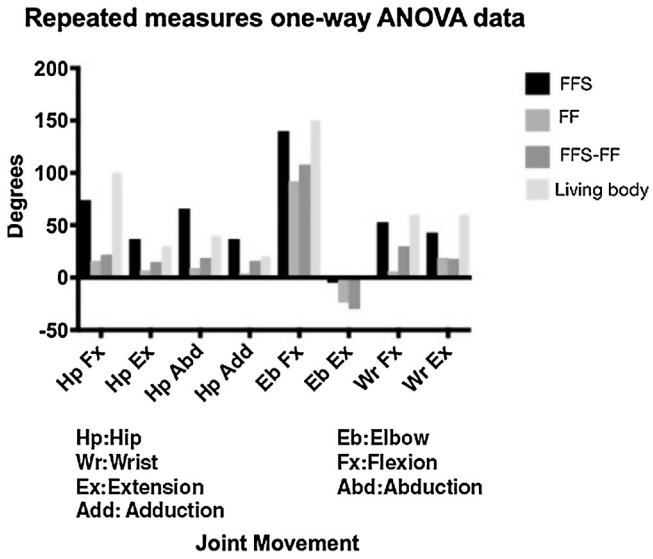

Fig. 3 – Goniometry measures for each joint movement.

Table 2 – CO_2 pressure needed to acquire adequate pneumoperitoneum.

Specimen	Initial pressure (mmHg)	Final pressure (mmHg)
FF	1	30[a]
FFS-FF	1	25
FFS	1	5

[a] No pneumoperitoneum was acquired at 30 mmHg where experiment stopped for security reasons.

specimen in which extensive flange release and a Nissen Fundoplication were performed after a diaphragmatic hernia was evident as an incidental finding.

Discussion

Previous studies have made comparisons between different preservation solutions, comparisons between Thiel solution and formaldehyde fixation are particularly relevant for this study since FFS and FFS-FF both share similar characteristics with Thiel's solution, and thus it can be regarded as the closest solution available in literature. A survey answered by medical students and physicians found that 98.5% of the students appreciated differences between corpses preserved with both techniques; physicians on the other hand, stated that Thiel-preserved bodies were a better choice on dissection and surgical training, since tissues offered a more realistic experience.[21]

Thiel solution properties are such that a corpse preserved for 37 months still retains ''great elasticity and contrast, getting really close to what could be found in a living body'', in which ''laparoscopic, arthroscopic, exploratory and surgical procedures can be performed as in a fresh body''.[26] Thiel gives corpses great flexibility, which makes them ideal for performing different educational activities. However they have a time limit and cannot be used for long.[27] Eight fresh and eight Thiel-preserved bodies were compared on an anesthesia department using ultrasound and found that nerve penetration and visibility were highly improved in Thiel-preserved corpses.[28] Thiel method can be used to recover formaldehyde damaged tissues, enhancing flexibility and color. After using Thiel solution ''muscles acquired a more realistic pink color, instead of the previous deep red they had before''.[29] Thiel preserved bodies have mainly been used in surgical simulation. Different studies show that Thiel preservation allows to perform many activities successfully such as intubations and minimally invasive procedures.[23]

Nonetheless, this technique has disadvantages such as its higher costs, requirement for vacuum conservation and training for handling, and while some authors report utility even after 37 months,[26] others report corpse's ''lifetime'' around a year for surgical simulation, and that the required chemicals and infrastructure are not readily available worldwide.[23] Thus, a modified Formaldehyde-free solution, similar in characteristics to Thiel's solution, is an alternative with lower costs that does not require any trained personnel and has good results in corpse preservation.

While FFS stands as a viable alternative for cadaveric preservation for both anatomy education and surgical simulation, the technique presents disadvantages when compared with Thiel. Due to the absence of nitrates in the solution, the color of the specimens is subpar, taking on sienna-like hues after successful preservation, unlike the life-like appearance of Thiel. Another disadvantage observed during the preservation process is that due to a very low concentration of formaldehyde, cerebral tissue is not preserved adequately and liquefies, thus barring simulation of neurosurgical procedures.

Our results showed that cadavers preserved with the FFS solution had a similar range of movements when compared to those reported to cadavers preserved with Thiel.[30] Flexibility is a crucial asset, since it allows better manipulation of cadaveric specimens, and aids in conserving the normal anatomy of all the structures.

Our study also showed that students preferred to work with FFS solutions rather than FF, since they were more flexible, had a better color, and avoided sensorial hassles. They also expressed their preference toward the body preserved with FFS-FF, which opens a door to the possibility of restoring and using corpses that were previously deteriorated and unsuitable for education.

FFS preserved specimens allowed for pneumoperitoneum and complex surgical simulation, including adhesion liberation and a complete Nissen Fundoplication, exhibiting flexibility, compliance, and tissue resistance, while previous experiences have enabled complex simulations such as thoracoscopy and arthroscopy, opening a new door for surgical simulation at a lower cost, with less hassles in the preservation process and with comparable specimen utility duration.

Histological examination of tissue preserved with FFS solutions revealed that morphologic details are accurately preserved, despite not being able to identify certain structures. This opens a door to future modifications that would allow Formaldehyde-free solutions to be used as preservation techniques for pathology laboratories. Other studies made

on Thiel's solution[31] also showed distortion on histological structures, suggesting that currently the only suitable solution for this type of research is FF.

Conflict of interest

None.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ciresp.2021.07.001.

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