Novel embalming solution for neurosurgical simulation in cadavers

Laboratory investigation

ARNAU BENET, M.D., JORDINA RINCON-TORROELLA, M.D., MICHAEL T. LAWTON, M.D., and J. J. GONZÁLEZ SÁNCHEZ, M.D., PH.D.

1Skull Base and Cerebrovascular Laboratory, Department of Neurosurgery, University of California, San Francisco, California; and 2Department of Neurosurgery, Johns Hopkins University, Baltimore, Maryland

Object. Surgical simulation using postmortem human heads is one of the most valid strategies for neurosurgical research and training. The authors customized an embalming formula that provides an optimal retraction profile and lifelike physical properties while preventing microorganism growth and brain decay for neurosurgical simulations in cadavers. They studied the properties of the customized formula and compared its use with the standard postmortem processing techniques: cryopreservation and formaldehyde-based embalming.

Methods. Eighteen specimens were prepared for neurosurgical simulation: 6 formaldehyde embalmed, 6 cryopreserved, and 6 custom embalmed. The customized formula is a mixture of ethanol 62.4%, glycerol 17%, phenol 10.2%, formaldehyde 2.3%, and water 8.1%. After a standard pterional craniotomy, retraction profiles and brain stiffness were studied using an intracranial pressure transducer and monitor. Preservation time—that is, time that tissue remained in optimal condition—between specimen groups was also compared through periodical reports during a 48-hour simulation.

Results. The mean (± standard deviation) retraction pressures were highest in the formaldehyde group and lowest in the cryopreserved group. The customized formula provided a mean retraction pressure almost 3 times lower than formaldehyde (36 ± 3 vs 103 ± 14 mm Hg, p < 0.01) and very similar to cryopreservation (24 ± 6 mm Hg, p < 0.01). For research purposes, preservation time in the cryopreserved group was limited to 4 hours and was unlimited for the customized and formaldehyde groups for the duration of the experiment.

Conclusions. The customized embalming solution described herein is optimal for allowing retraction and surgical maneuverability while preventing decay. The authors were able to significantly lower the formaldehyde content as compared with that in standard formulas. The custom embalming solution has the benefits from both cryopreservation (for example, biological brain tissue properties) and formaldehyde embalming (for example, preservation time and microorganism growth prevention) and minimizes their drawbacks, that is, rapid decay in the former and stiffness in the latter. The presented embalming formula provides an important advance for neurosurgical simulations in research and teaching.

Key words • embalming • neurosurgery • simulation • anatomy

Neurosurgery is one of the most challenging surgical specialties because it deals with the most complex and fragile organ in the human body—the brain—and because it requires a combination of precise technical skills, experience in the surgical setting, and superb knowledge of anatomy. Surgical simulation using a cadaveric human head is one of the most valid strategies for neurosurgical research and training because it provides the closest approximation to a live surgical procedure with true human anatomy.

Several models for neurosurgical training have been
described in the literature. The main goal of anatomical cadaveric processing is to achieve the most realistic model possible. There have been several major breakthroughs in this field. However, none of these cited studies provides advances in cadaveric embalming methods to maintain the physical properties of a living brain while preserving the brain specimen from decay.

The major physical properties to be considered when addressing embalming research are brain stiffness, preservation time, and biohazard safety. Two common processing techniques used in cadaveric neurosurgical simulations are cryopreservation (unembalmed) and formaldehyde-based preservation. Cryopreserved specimens provide optimal brain stiffness but have a short preservation time and are considered biohazardous. Formaldehyde-based embalming formulas are the standard for long preservation of specimens. However, formaldehyde substantially increases brain stiffness, making retraction and surgical simulation very difficult. Additionally, many studies have reported that long-term exposure to high airborne formaldehyde concentrations in the laboratory is hazardous. In 2006, the International Agency for Research on Cancer (IARC) and the US Environmental Protection Agency classified formaldehyde as a probable human carcinogen.

We have customized an embalming formula for neurosurgical simulations that enhances brain compressibility and enables retraction while preventing microorganism growth and brain decomposition. This cadaveric embalming formula also decreases potential chemical biohazards to meet the IARC recommendations for laboratory safety. In the present work, we studied the properties of our customized formula and compared its use with standard postmortem processing techniques—cryopreservation and formaldehyde embalming—in a sample of cadaveric specimens. We also analyzed the applications of each technique to neurosurgical training and research and provide recommendations on specimen preparation for neurosurgical simulations.

**Methods**

To study the properties of 3 cadaveric processing techniques (cryopreservation, formaldehyde-based embalming, and customized-formula embalming), 18 human specimens (age range at death 50–95 years) were prepared for surgical simulation. Donors with premorbid conditions of the CNS were excluded from our study. Specimens in the embalmed groups (formaldehyde and custom) were kept immersed in their respective embalming fluids for a mean time of 8 months (range 2 weeks–1 year) before the experiment. Six cryopreserved and 6 formaldehyde-embalmed specimens were prepared according to conventional processing techniques for neurosurgical research. Six additional specimens were prepared using our customized embalming solution. A standard pterional approach was performed in all specimens to compare brain compressibility, retraction profile, and preservation time. One MR image of 2 specimens in each group was obtained. One customized specimen was also prepared to test the feasibility of bleeding simulations.

**Head Preparation**

All heads were prepared for optimal neurosurgical simulation. The neck was sectioned at vertebrae C5–7 to provide good exposure of cervical vessels and preserve the cervical spinal cord. Common carotid and vertebral arteries along with jugular veins were identified and isolated. Minimal sharp dissection was performed around vessels to prevent undesired rupture of deep arteries and veins, which could cause leakage during silicone injection. Cervical arteries and jugular veins in the embalmed groups were cannulated according to previously described methods. Arterial and venous systems were cleaned using saline solution until contralateral outflow was clear. This procedure was repeated bilaterally on each cannulated vessel, alternating arterial and venous irrigation. Once all blood clots were cleared from external and internal vascular systems (carotid and vertebral arteries and jugular veins), the specimens were randomly divided into the customized and formaldehyde groups. Because of their fast decay time, cryopreserved specimens were not cannulated or injected to maximize their experiment time.

**Embalming Procedures**

Customized and standard formaldehyde embalming solutions were used for comparative analysis. One-half liter of fixative was perfused through common carotid and vertebral arteries (200 ml) and jugular veins (300 ml) in each head. The customized mixture was prepared in the laboratory using the following formula: ethanol 62.4%, glycerol 17%, phenol 10.2%, formaldehyde 2.3%, and water 8.1% (Fig. 1). A conventional 10% formaldehyde solution was used for the formaldehyde group. All embalmed heads were immersed in a 1:10 dilution of the respective embalming fluid and stored at 5°C for at least 2 days before silicone injection. Cryopreserved specimens were frozen at postmortem Day 1–5 at −15°C to −20°C and thawed for approximately 12 hours before proceeding with the surgical simulation.

**Vascular Silicone Injection**

Arterial systems were injected with red silicone, and venous systems with blue silicone (Fig. 2). The arterial system—common carotid and vertebral arteries—was processed first to secure filling of the distal and small thalamoperforating arteries. Common carotid arteries were bilaterally injected until vertebral artery colored outflow was observed. Bilateral vertebral artery injection was then performed until the arterial system was fully injected. Finally, the arterial system was clamped except for 1 carotid artery, which was used to increase arterial pressure to force small-caliber vessels to fill. Upon completion of arterial silicone injection, jugular veins were processed using the same injection principles.

**Comparative Analysis**

The durability and retraction (stiffness) properties of specimens treated with our customized formula were compared with the properties of specimens treated with cryopreservation and formaldehyde-based preservation. A standard pterional craniotomy was performed on each
Novel embalming solution for surgical simulation

The dura mater and arachnoid membranes were carefully removed, and the temporal lobe was gently retracted dorsally to simulate a subtemporal approach. Retraction profiles were measured using an intracranial pressure transducer and monitor (Integra Camino parenchymal intracranial pressure monitoring kit) inserted 8 mm into the inferior temporal gyrus, 4 cm posterior to the temporal pole (Fig. 3). Pressure measurements were recorded before temporal lobe retraction and at the tissue retraction limit. This limit was set at the highest, most retractile pressure before tissue damage and was dependent on the retraction profile of each specimen. The optic and oculomotor nerves, supraclinoid internal carotid artery, anterior clinoid process, and tentorium were used as surgical landmarks to compare subtemporal surgical exposures among the processing techniques.

The total retraction surface was also measured. Ten pins were inserted along the cortex surface and registered as stereotactic points using a surgical navigation system (Stryker Nav3). The pins remained in the same cortical surface location throughout the experiment. Stereotactic coordinates were obtained from each pin by touching it with the navigation probe at resting state and at the tissue retraction limit (Fig. 3 right). Surface areas were calculated from the stereotactic coordinates using dedicated software (Surface Area Calculator, BitWise Ideas Inc.) and recorded in a spreadsheet for statistical analysis. Retraction surface was obtained by subtracting the area at retraction limit from the area at resting state.

We also sought to study the durability (preservation time) of specimens. Two specimens from each group were prepared and continuously exposed to laboratory working conditions for 2 days. We studied changes in tissue consistency, color, and decay to compare specimen conditions at 2-hour intervals for 2 consecutive days. An itemized observational study spreadsheet was completed for the duration of the experiment, and consistency, color, and overall appearance were recorded as dichotomous variables (1 = changes observed, 2 = no changes observed). Subjective appreciations (odor and texture) were also recorded and analyzed after the experiment. To complement the observational study, we administered a blinded survey to a sample of 4 neurosurgery residents and 2 attending neurosurgeons to evaluate the best specimen group after the experiment ended. The volunteers were asked to “please rate hierarchically the images in the attached figure as to their similarity to the real brain in terms of color and texture.”

Data were collected and descriptive statistics was performed using SPSS Statistics Desktop, version 21.0 (IBM Corp.). The mean retraction pressures, retraction surfaces, and durability lapsing times were compared using independent Student t-test analysis. A p < 0.05 was considered statistically significant.

Imaging and Postprocessing Techniques

Radiological studies are very important in neurosurgical simulation and research; therefore, 3-T T1-weighted FLAIR MRI was performed on 2 heads from each group on the same day as specimen processing. The radiological images were used to compare the quality and preservation of internal nuclei, cortex, white matter, and the whole brain.
encephalon before starting the surgical simulations. In 1 case, a cryopreserved specimen received on postmortem Day 8 was scanned. Although this specimen provided clear radiological evidence of the decay process in cryopreserved specimens, it was excluded from the morphometric study.

The bleeding model for neurosurgical simulation was prepared and tested in 1 customized specimen as described elsewhere.1,16

Results

All formaldehyde and customized specimens were completely embalmed. The cortex surface along the watershed area was uniformly embalmed. Surgical simulation experiments were successfully performed in all specimens included for morphometric study. One cryopreserved specimen with advanced decay on MRI was excluded from the morphometric experiment sample and replaced.

Retraction Profiles

The subtemporal approach was completed in all specimens. Retraction profiles (retraction pressure and surface) of the customized and cryopreserved specimens were very similar to each other and clearly better than that of the formaldehyde specimen. The subtemporal approach provided equivalent exposure of the entire incisural space and cavernous sinuses in cryopreserved and customized specimens. However, only the tentorium and superior cerebellar artery in the middle incisural space were exposed in the formaldehyde group. At maximal retraction, deep plane maneuvering and dissection around the parasellar region were identical in the customized and cryopreserved groups and very difficult in the formaldehyde group.

Retraction Pressure. Retraction pressure studies were performed to assess the brain compliance and retraction capabilities of each group (Table 1). Overall mean retraction pressures were highest in the formaldehyde group and lowest in the cryopreserved group. At the maximal retraction point, our customized formula provided a mean retraction pressure almost 3 times lower than formaldehyde (36 ± 3 vs 103 ± 14 mm Hg, p < 0.01) but slightly higher than cryopreservation (36 ± 3 vs 24 ± 6 mm Hg, p < 0.01; Fig. 4).

Retraction Surface. Retraction surface was calculated to assess brain stiffness during a standardized neurosurgical procedure and to compare the surgical area gained during retraction. There was no statistical difference between the customized and cryopreserved groups (p = 0.13), but the retraction area of the customized group was almost 4 times larger than that of the formaldehyde group (1.44 ± 0.4 vs 0.46 ± 0.1 cm², p < 0.01; Fig. 5). These retraction profiles provided different access to the incisural space and posterior fossa structures.

Specimen Condition

We have observed that flushing the vascular system with isotonic saline solution instead of tap water both prevents brain and tissue edema and provides optimal cleaning of blood clots. In our experience, the low osmolality of the tap water produced massive edema in all cases. It was prevented by the use of an isosmolar saline solution instead of tap water. In addition to using saline solutions, we preferred to repeatedly manually inject at low pressure to cleanse the vessels. This procedure increased the overall quality as relates to color, texture, brightness, and clarity of all specimens, but especially those treated with our customized formula. Silicone injection was completed in all specimens regardless of the embalming method. All thalamoperforating arteries and other distal vessels were fully injected, and no subarachnoid silicone leak was observed. The customized specimens, as compared with the formaldehyde-preserved specimens, exhibited color and texture closer to those in real life.

Using only 2.3% of formaldehyde in the customized formula, we reduced the formaldehyde content by 78%, as compared with standard embalming solutions. Texture and color were similar for the cryopreserved and customized specimens at the time of brain exposure (Fig. 6A and B). However, formaldehyde-fixed specimens were stiffer and slightly darker (Fig. 6C). Degradation of tissue consistency and color was observed in the cryopreserved specimens after continuous exposure to the working environment for 4 hours, whereas customized and formaldehyde-preserved specimens maintained their properties.

<table>
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<th>Group</th>
<th>Measure</th>
<th>No. of Specimens</th>
<th>Min</th>
<th>Max</th>
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<td>18</td>
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* Control pressure, pressure tissue break, and retraction area were measured in each specimen.
Novel embalming solution for surgical simulation

Fig. 4. Retraction profile graph of the study groups. The formaldehyde group had the highest mean pressure at tissue break (over 100 mm Hg, bar) and provided the least retraction area (0.5 cm², black line). The customized and cryopreserved groups had similar retraction profiles. The customized group offered almost 3 times less resistance to retraction than the formaldehyde group. The cryopreserved group provided the largest retraction surface (2.3 cm², black line) with the lowest retraction pressure (24 mm Hg, bar).

Fig. 5. Box plot of the retraction area for each group. The formaldehyde specimens provided less retraction area than the other groups. There was no statistical difference between the customized and cryopreserved specimens, which had more variability and 1 outlier.

Fig. 6. Photographs showing specimen conditions. The customized (A), cryopreserved (B and D), and formaldehyde (C) groups were exposed to continuous dissection for 48 hours. The color and texture of the customized and cryopreserved specimens were similar. Specimens in the formaldehyde group were darker in color and stiffer in texture than those in the other groups. All specimens were kept wet during the experiment. The customized and formaldehyde specimens maintained initial conditions, and no changes were noticed. In contrast, the cryopreserved specimens showed advanced signs of decay at Hour 40 (D).

throughout the entire experiment. While evident signs of brain liquefaction were noted in the cryopreserved specimens after working Hour 40 (Fig. 6D), consistency and color were preserved in both customized and formaldehyde-fixed specimens. The customized specimens were consistently rated favorably, as compared with the formaldehyde and cryopreserved specimens at the end of the experiment. Changes in consistency were observed in the cryopreserved group from Hour 2. All the observational variables—color, consistency, and overall appearance—were consistently rated 1 (change observed) from Hour 4 throughout the experiment. Sporadic changes in color and overall appearance were noted in both embalmed groups, which resolved with tissue hydration.

Formaldehyde vapors emanating from the formaldehyde-fixed specimens were noticeable in the working environment immediately after positioning the head for dissection. Placing the specimens under running water for 15 minutes before dissection diluted the vapors. Unpleasant, aggressive odor from decay and microorganism growth prevented researchers from continuing dissection of cryopreserved specimens at working Hour 7. In contrast, no microorganism growth or brain decay was obvious in the embalmed specimens during the study.

Imaging and Postprocessing Techniques

Overall, radiological studies of the customized specimens were similar to and better than those of the cryopreserved group, whereas the formaldehyde group imaging was notably worse in terms of the level of anatomical detail and MRI signal contrast. Although T1-weighted MRI of the customized group showed optimal definition of the cortex, sulci, and white matter, some artifacts and hyperintensities were observed randomly along the internal nuclei (Fig. 7B and E). The best images of the internal nuclei were obtained from the cryopreserved group (Fig. 7C and F). The embalmed specimens had better tissue preservation than the cryopreserved specimens, which showed signs of brain shrinking and frontal pneumocephalus. At postmortem Day 8, the cryopreserved specimens showed signs of advanced decay compared with the embalmed specimens (Fig. 8).

The bleeding model for neurosurgical simulation de-
scribed by Aboud et al. was also tested in 1 customized specimen. Brain movements encompassing arterial beating were observed at a normal arterial pressure resembling live surgery. Hemorrhage from deliberate arterial rupture permitted deep surgical field hemostasis simulation. Gentle retraction of the sylvian fissure together with careful sharp dissection and hemostasis allowed exposure of the middle cerebral artery in a highly simulative surgical scenario.

**Discussion**

Results of this study show that the customized embalming formula provides an optimal brain retraction profile, long use time, and low biohazard risk for neurosurgical simulation in cadavers. Overall, the customized group provided the best feature combination for cadaveric neurosurgical research. We found that specimens embalmed with our customized formula offered 3 times less retraction pressure and 4 times larger surgical retraction area than the specimens in the formaldehyde group. When surgical simulation was performed, we obtained best access to surgical landmarks and maneuverability in the customized and cryopreserved groups. Nevertheless, cryopreserved specimens had a very limited working time because of fast decay (4 hours for research purposes). Moreover, radiological studies showed that the customized and cryopreserved groups provided better anatomical detail than the formaldehyde group, although random hyperintense artifacts were identified in the customized specimens. Furthermore, we demonstrated that surgical bleeding simulation is feasible in our custom-embalmed specimens.

The key concept when choosing a processing technique is to know exactly the dissection objectives and requirements of the proposed work and to have a thorough understanding of the different properties of the available preservation methods. Many specimen-processing techniques are available for laboratory use, and each has specific advantages and disadvantages.

Solutions containing large formaldehyde concentrations have been used as the standard in specimen preservation because they offer long-term preservation and prevent microorganism growth. Despite these desired properties, however, formaldehyde remains a suboptimal fixation method for neurosurgical simulation because the brain stiffness it produces makes retraction very challenging. Nonetheless, formaldehyde embalming has provided researchers with an excellent processing method for gross anatomy teaching and white matter dissection. Formaldehyde-related brain hardening combined with sequential freezing causes axons to separate from each other, thus facilitating the dissection of fine fiber bundles. In contrast, color distortion is a well-known drawback that has an important impact on the overall quality of the illustrative work in terms of more lifelike and better contrast. White, pallid cortical and subcortical structures are far

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**Fig. 7.** Radiological studies of specimens. Axial and sagittal 3D T1-weighted MR images of the formaldehyde (A and D), customized (B and E), and cryopreserved (C and F) specimens revealed better overall results in the customized group. The cortex and subcortical nuclei were best identified in the cryopreserved group and were not identified in the formaldehyde group. The cryopreserved specimen showed signs of decay and pneumocephalus.

**Fig. 8.** Coronal 3D T1-weighted MR images of the customized (left) and cryopreserved (right) specimens at postmortem Day 8. The cryopreserved specimen showed evident signs of decay—Mount Fuji sign of pneumocephalus with its superior surface tethered to the superior sagittal sinus.
Novel embalming solution for surgical simulation

from natural brain color and can sometimes be difficult to contrast with cranial nerves during surgical simulations.

It has been widely accepted that cryopreservation (not embalmed) is the cadaveric processing technique that least modifies the biological properties of brain tissue. Nonetheless, the natural appearance of the brain only lasts for a very limited time. At working Hour 4 (around postmortem Day 2–5), specimens are no longer useful for neurosurgical simulation research, as liquefaction prevents reliable morphometric analysis and tissue distortion becomes evident. This fast degradation is a major drawback during hands-on courses or other teaching activities where an extended period of time but reliable anatomy and surgical maneuverability are needed to understand and practice the different surgical approaches. Another significant drawback of cryopreserved specimens is biohazard safety. Because unembalmed specimens lack germicidal agents, uncontrolled microorganism growth can occur early in the dissection process. However, unembalmed specimens continue to be used in procedure demonstrations and educational workshops because they used to be the only means of obtaining features most similar to those of a living brain in the operating room (that is, the possibility of brain retraction). In our opinion, our customized embalming technique opens a new possibility for brain retraction and provides brain features similar to those of cryopreserved specimens while also offering a more cost-effective and safer option.

The customized embalming formula takes advantage of the best formaldehyde properties—long preservation time and microorganism growth prevention—combined with a retraction profile and physical properties similar to those attained with cryopreservation. Moreover, a substantial reduction in the formaldehyde concentration is biohazard safety. Because unembalmed specimens lack germicidal agents, uncontrolled microorganism growth can occur early in the dissection process. However, unembalmed specimens continue to be used in procedure demonstrations and educational workshops because they used to be the only means of obtaining features most similar to those of a living brain in the operating room (that is, the possibility of brain retraction). In our opinion, our customized embalming technique opens a new possibility for brain retraction and provides brain features similar to those of cryopreserved specimens while also offering a more cost-effective and safer option.

The customized specimens provided an unparalleled surgical scenario when using the bleeding model. Our formula provided good brain retraction capabilities, allowing surgical exposure, vascular dissection, and bleeding control in a very realistic scenario.

Biohazard Risk and Health Standards

Health protection is a primary concern and must be seriously considered in a surgical simulation laboratory. Although general protection standards (physical barriers) must always be used, special measures tailored to embalming chemicals are necessary to provide a safe workplace.

Neurosurgical simulation, especially for research and illustrative purposes, takes time, and a continuous dissection process demands a high level of concentration to perform meticulous and precise anatomical exposures. In our study, microorganism growth together with unpleasant aggressive odor and evident signs of tissue decomposition forced researchers using cryopreserved specimens to finish dissection prematurely. This situation can be frustrating because dissection targets and research objectives are most often achieved in the last steps of dissection work. Moreover, intense continuous work in a crowded workplace (for example, hands-on workshops) lowers the ventilation rate and increases room temperature, thus increasing the decay rate in cryopreserved specimens and airborne formaldehyde particles when using formalde-
hyde-preserved specimens. Maintaining these circumstances over a prolonged time could result in a harmful biohazard situation. Therefore, the federal government and the state of California have established permissible exposure limits for chemical exposures in the workplace.

Airborne formaldehyde is the most harmful volatile chemical among those included in the present study and has the strictest biohazard security control codes. Many institutions and government regulations have considered it to be a carcinogenic agent. Although formaldehyde is still needed to prevent microorganism growth (especially fungi), formaldehyde content in the customized embalming fluid described here was almost 80% less than in a conventional formaldehyde mixture. Furthermore, customized specimens provided a less aggressive and contaminated environment than the formaldehyde and cryopreserved specimens. Researchers noticed no airway or mucosal irritations when working with either the customized or cryopreserved group. However, slight to mild oropharynx and eye mucosal irritation was present when using formaldehyde specimens.

Fixative and germicidal properties of the customized embalming formula are provided mainly by phenol and ethanol. Although phenol is considered to be more hazardous than ethanol, its toxic effects are limited to skin contact. Furthermore, IARC evaluation of phenol concludes that it is “not classifiable as to its carcinogenicity to humans.” Thus, airborne biohazard particles in the laboratory are drastically reduced when using a specimen preserved with our customized embalming formula. However, pertinent physical barriers as well as a high ventilation rate in the workplace are strongly recommended while manipulating specimens or the embalming mixture.

Study Limitations

Although most laboratories do not disclose their embalming formulas, we chose to share our customized formula for neurosurgical simulations and evaluated its properties through a comparative analysis. Embalming options other than those included in this study are also available on the market. Moreover, there are tissue softeners that presumably reduce brain stiffness by removing formaldehyde (fixative) from the specimen. Although it is beyond the scope of this study to compare all of the available processing techniques, the proposed customized embalming formula is easier to produce than other special chemicals and easier to use than the successive embalming and softening steps or complex measurements in other techniques.

Conclusions

Evidence in the present study supports the use of specimens embalmed with a customized formula for cadaveric neurosurgical simulations, especially for work times lasting more than 4 hours. Moreover, this embalming technique provides a better balance of the major physical properties to be considered in embalming research—brain stiffness, preservation time, and biohazard safety—than cryopreserved or classic formaldehyde-based processing methods.
Novel embalming solution for surgical simulation

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