Original Article

Usefulness of cadaver embalming solutions as alternatives to formalin in veterinary surgical training

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Abstract

Veterinary surgical training with live animals is inevitable for skill development. However, using a live animal is impractical owing to cost and ethical standards. Thus, well-embalmed life-like cadavers should be used. This study aimed to assess the usefulness of cadaver embalming in surgical training using two alternatives to formalin solution (FS)—Thiel's solution (TS) and saturated salt solution (SS). Four groups, each comprising three Beagles, used FS, TS, and SS for cadaver embalming, and one group served as control. Skin elasticity, range of motion (ROM), tissue color, and life-like conditions during surgical procedures were evaluated at one-month intervals for a total of four months. Skin elasticity in the TS group was more similar to that of the control group in the first and second months, and to that in the SS group from the first to the third month, while that of the FS group was lower than that of the control group in the second and fourth months ($P < 0.05$). ROM in the TS group showed no difference than that in the control group ($P > 0.05$), while ROM in the FS group was lower than that in the control group ($P < 0.05$). Overall tissue color in the TS group was not different from that in the control group until the third month ($P > 0.05$). On evaluation of life-like conditions during surgical training, TS group cadavers tended to be superior to those in other groups in the first month ($P < 0.05$), but gradually decreased, and in the fourth month, the score was lower than those of the SS group ($P < 0.05$). TS cadavers showed the most life-like conditions with regard to elasticity, color, ROM, and surgical aspects and thus were suitable for surgical training within three months. This study is clinically relevant in minimizing animal sacrifice by effective and long-term conservation of quality cadavers.

Keywords: canine cadaver embalming, formalin solution, saturated salt solution, Thiel's solution, veterinary surgical skill training

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Introduction

Veterinary surgical techniques evolve constantly, and the mastery of surgery requires a combination of accurate knowledge and manual skill to perform delicate and sophisticated surgeries (Eisma *et al*., 2011; Hölzle *et al*., 2012). Expertise in dissection, tissue handling, and suture techniques is difficult to obtain only by training in the operating room, and theoretical training alone, without hands-on practice, is difficult (Reed *et al*., 2009). Therefore, the practice of using live animals is inevitable for education and skill development. However, the use of live animals in training is impractical owing to cost and ethical issues. Today, the recognized animal experiments ethical standards are those provided by Russell and Burch's principle of the 3Rs—Replacement, Reduction, and Refinement (Russel and Burch, 1959).

According to the 3Rs, the number of animals sacrificed should be as minimal as possible. After live animals, the next best alternative for use in veterinary surgical practice is fresh or fresh-frozen cadavers because of their life-like color, softness, and pliability (Macchi *et al*., 2003). Tissue properties that are as realistic as possible is the most important characteristic of preserved cadavers for surgical training (Hayashi *et al*., 2014). Unfortunately, since putrefaction occurs rapidly after death, fresh or fresh-frozen cadavers are not suitable for long-term preservation; thus, the possibility of embalming cadavers to mimic life-like conditions is essential (Brenner, 2014).

Formalin is a typical solution for identifying anatomical structures. However, formalin fixation results in dehydration of organs and tissue, stiffness of joints, and discoloration of tissue (Balta *et al*., 2015). Therefore, many studies have sought to identify more suitable alternatives to formalin (Eisma*et al*., 2011; Haizuka *et al*., 2018; Hayashi *et al*., 2016; Turan *et al*., 2017). Thiel's solution (TS) and saturated salt solution (SS) are appropriate for surgical practice because they keep cadavers pliable (Hayashi *et al*., 2014, Hayashi *et al*., 2016, Thiel, 2002, Thiel, 1992). This study investigated the usefulness of TS and SS cadaver embalming solutions by measuring life-like conditions to determine the best formalin-alternative to ensure surgical suitability of cadavers for veterinary surgical training.

Materials and Methods

Animals and ethics: This study was conducted with approval of the Institutional Animal Care Use Committee of Konkuk University (approval no. KU 18132).

Twelve Beagles, weighing 8.9–11.9 kg, were purchased from Raon Bio Inc. (Gyeonggi-do, Korea) and euthanized. They had no remarkable findings on overall physical examination. These 12 cadavers were stratified into 3 groups, and each group was evaluated after embalming once a month for a duration of 4 months under similar conditions. Each group consisted of three cadavers embalmed in formalin solution (FS), TS, SS, and one group served as control. In the control group, the values were measured immediately after euthanasia without any embalming.

Embalming methods for cadavers: Each fixative solution was prepared a described previously (Hayashi *et al*., 2014; Thiel, 2002; Thiel, 1992). FS contained 5% formaldehyde, SS had 2.68% formalin, and TS contained 2.13% formalin. The detailed composition of each solution is listed in Table 1. The dogs were intramuscularly anesthetized with 10 mg/kg of zolazepam and tiletamine (Zoletil® 50; Virvac, Carros, France). Under general anesthesia, the dogs were positioned in dorsal recumbency, and ventral neck region was incised to expose the carotid artery. Exsanguination was performed through a cannula inserted into right common carotid artery, and the embalming fluid was infused via the cannula. The total volume of the embalming solution required for each cadaver was up to 1.5 *L* per 10 kg, and 50 *mL* of the solution was injected into any area that appeared to be insufficiently infused with the embalming solution, such as the abdominal cavity and the thoracic cavity. After injection of the embalming solution, the exposed vessels were ligated. After the embalming process, each cadaver was packed in a plastic bag and stored at 4 °C until the time of the first measurement. After each measurement was performed, the cadavers were returned to 4 °C storage until the next measurement timepoint.

Measurement of skin elasticity: Skin elasticity was measured with an ultrasonic vibration system using a Triplesense® (MORITEX Co, Saitama, Japan). The sensor vibrates at a specific frequency that changes upon contact with the skin, thus reflecting its elasticity; the greater the change in frequency, the more elastic the skin is. The average measured elasticity was calculated from five measurements in the trunk area (skin of the latissimus dorsi located at the caudal part of the scapula); flank (skin of the transverse abdominal muscle located at the cranial part of the sartorius muscle); and the hindlimb (skin of the lateral part of the biceps femoris muscle). The lowest value of skin elasticity was 0 and the highest value was 99. Skin elasticity was measured at monthly intervals for four months.

Range of motion (ROM) of joint: ROM was measured using a 15-cm goniometer with one-degree graduations. Extension was defined as the joint stretched to the maximum extension, while flexion was defined as the joint bent to the maximum flexion. To measure the joint joining angle, the center of the goniometer was placed at the center of the joint motion with one side placed on the long axis of the distal bone and the other placed on the long axis of the proximal bone. The ROM values of the extension and flexion angles in the shoulder, elbow, carpal, hip, stifle, and tarsal joints were measured.

Measurement of muscle and liver color: Muscle and liver tissue colors were evaluated using a dual-beam spectrophotometer (SpectraTrend® HT; Hunter Associates Laboratory, Inc. Virginia, USA). The parameters of Commission on Illumination (French Commission internationale de l'éclairage [CIE]) L*a*b* color space was used for color measurement; the CIEL color space is shown in Figure 1. L* stands for luminosity, which indicates darkness or lightness on a 0–100 scale from black to white; a* indicates redness or greenness, and positive values represent redness and negative values represent greenness; and b* represents yellowness as denoted by a positive value, while blueness is denoted by a negative value.

The biceps femoris muscle and liver of each cadaver were dissected and used for color measurement. Since the color of the liver and muscle represents a unique value, similarity with the control group indicates the degree of life-like conditions of each organ. The colors were measured three times for each measurement, and the mean values were used for evaluation. The color was measured once a month for four months.

Table 1 Composition of three embalming solutions

Formalin solution		Saturated salt solution		Thiel's solution	
Solution formula	**Amounts**	**Solution formula**	**Amounts**	**Solution formula**	**Amounts**
40% Formalin	0.25 *l*	Sodium chloride	4.0 Kg	**1. Stem solution A**	
Ethanol	1.50 *l*	40% Formalin	0.14 *l*	4-Chloro-3-methlphenol	26.40 g
Phenol	0.10 *l*	Isopropyl alcohol	0.80 *l*	Propylene glycol	0.264 *l*
Glycerol	0.10 *l*	Phenol	0.04 *l*	**2. Stem solution B**	
Water	3.05 *l*	Glycerol	0.10 *l*	Ammonium nitrate	1.0 Kg
		Water	4.52 *l*	Hot water	1.6 *l*
				3. Stem solution C	
				Boric acid	148 g
				Potassium nitrate	248 g
				Hot water	2.0 *l*
				4. Stock solution	
				Stem solution A	0.264 *l*
				Stem solution B	1.60 *l*
				Stem solution C	2.00 *l*
				Propylene glycol	1.48 *l*
				Hot water	1.32 *l*
				5. Final solution	
				Stock solution	6.664 *l*
				Sodium sulfite	320 g
				40% Formalin	0.16 *l*
				Morpholine	0.12 *l*
				Ethanol	0.52 *l*
Total	5.00 *l*	**Total**	5.00 *l*	**Total**	7.544 *l*

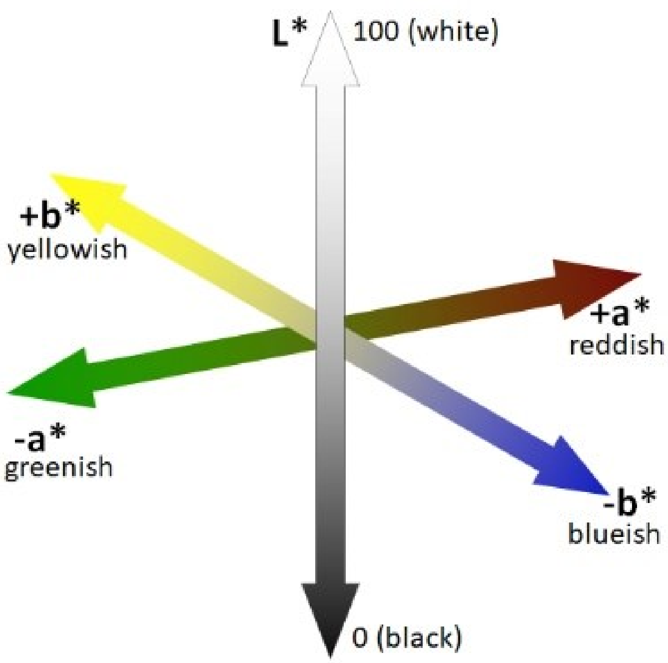

Figure 1 **Commission on Illumination L*a*b* color space.** L* stands for luminosity indicating darkness or lightness, on a 0 - 100 scale from black to white. a* indicates redness or greenness, positive value meaning redness and negative value meaning greenness. b* indicates yellowness of positive value and blueness of negative value.

Evaluation of suitability for surgical skill training: Surgeons performed five surgical procedures, including endotracheal tube intubation, skin flap surgery, lateral ear canal resection, cystotomy, and enterotomy on each embalmed cadaver. Each surgeon had previous experience in the respective surgeries, and each performed comparative evaluation of each surgical procedure for the three groups (FS, TS, and SS). The surgeons conducted blinded postoperative evaluations (they did not know which embalming solution had been used). The degree of life-like conditions of cadavers was evaluated postoperatively using a postoperative questionnaire with a score of 1–10. The surgical procedures were performed in a routine manner based on the Small Animal Surgery (Fossum *et al,* 2018).

Statistical analysis: Statistical analyses were performed using IBM SPSS Statistics (IBM, Armonk, NY) for Windows. The results were analyzed using one-way analysis of variance, followed by Tukey's post-hoc test for multiple comparisons of cadaver skin elasticity, ROM, color, and surgical suitability; these values were compared with those of the control group as well as between the different groups. A P-value of < 0.05 indicated statistical significance.

Results

Skin elasticity: Skin elasticity results for the trunk, flank, and hindlimb during the first to fourth month of embalming are presented as mean ± standard deviation (SD) in Table 2. The skin elasticity of all anatomical sites in the FS group was significantly lower than that in the control group at the second, third, and fourth months ($P < 0.05$). All sites in the TS group had significantly lower values of kin elasticity than those of the control group at the third and fourth months ($P < 0.05$). All sites in the SS group had significantly lower values of skin elasticity than those of the control group at the fourth month ($P < 0.05$).

Evaluation of ROM of joints: The differences between the extension and flexion ROM of the shoulder, elbow, carpal, hip, stifle, and tarsal joints during the first to fourth month are expressed as mean ± SD in Table 3. The FS group had significantly lower ROM values than those of the control group for all joints at all measured time points ($P < 0.05$). The difference in ROM in the TS group was not significantly different from that in the control group for all joints and at all time points ($P > 0.05$).

Color assessment: The mean ± SD color measurement values of L*, a*, and b* in the biceps femoris muscle and liver during the first to fourth months are described in Table 4. Only a small difference in color was found between live animals and the control group, thus indicating a similarity in color between the two groups. Figure 2 shows the gross appearance of the liver at the second month of evaluation.

The overall color values in the TS group were not significantly different from those of the control group until the third month ($P > 0.05$); SS group had overall color values that were most similar to those of the control group at the fourth month ($P > 0.05$), and FS group showed the most significant difference in the color values, relative to that of the control group, from the first to the fourth month ($P < 0.05$).

Assessment of surgical suitability: The mean ± SD scores of the life-like condition for surgical suitability are described in Table 5. The FS group had the lowest score in all tests ($P < 0.05$), except cystotomy and intestinal resection at the fourth month. TS-embalmed cadavers tended to be superior to other embalmed cadavers in the first month ($P < 0.05$), but the surgical suitability score gradually decreased, and was lower than that of SS-embalmed cadaver in the fourth month ($P < 0.05$).

Table 2 Skin elasticity of trunk, flank, and hindlimb of control and three embalming solution groups (mean ± SD)

		Time (month)			
Sites	**Group**	**1st month**	**2nd month**	**3rd month**	**4th month**
Trunk	Control			94.5 ± 0.6	
	FS	93.3 ± 1.1	92.7 ± 0.3†	90.9 ± 0.3†a	91.0 ± 0.3†a
	TS	94.4 ± 0.6	93.7 ± 0.1	86.5 ± 1.0†b	84.6 ± 0.7†b
	SS	93.7 ± 1.0	94.1 ± 0.9	93.9 ± 2.5a	91.3 ± 0.6†a
Flank	Control			94.9 ± 0.1	
	FS	93.4 ± 0.5	92.5 ± 0.4†	92.3 ± 0.9†a	91.9 ± 0.7†a
	TS	95.1 ± 1.1	94.1 ± 0.6	88.7 ± 0.7†b	85.6 ± 1.1†b
	SS	93.7 ± 1.5	93.4 ± 1.1	93.4 ± 1.4a	91.0 ± 0.5†a
Hindlimb	Control			94.5 ± 0.5	
	FS	93.3 ± 1.7	91.3 ± 1.7†	91.5 ± 0.5†b	91.3 ± 0.2†a
	TS	94.4 ± 0.9	94.0 ± 0.9	87.6 ± 0.7†c	85.9 ± 0.8†b
	SS	93.6 ± 0.7	93.5 ± 0.8	94.5 ± 0.5a	91.5 ± 0.2†a

FS = formalin solution; TS = Thiel's solution; SS = saturated salt solution.
P values of < 0.05 indicated statistically significant.
[abc] values with different letters in each month of each sites indicate significantly different ($P < 0.05$).
† indicates significantly lower than control group ($P < 0.05$).

Table 3 Range of motion on shoulder, elbow, carpal, hip, stifle, tarsal joint of control and three embalming solution groups (mean ± SD)

		Time (month)			
Joints	**Group**	**1st month**	**2nd month**	**3rd month**	**4th month**
Shoulder	Control	78 ± 2			
	FS	9 ± 2[†c]	9 ± 7[†c]	11 ± 5[†c]	11 ± 6[†c]
	TS	83 ± 2[a]	82 ± 3[a]	82 ± 3[a]	76 ± 5[a]
	SS	55 ± 7[†b]	51 ± 5[†b]	48 ± 4[†b]	51 ± 3[†b]
Elbow	Control	89 ± 5			
	FS	65 ± 9[†c]	52 ± 13[†b]	51 ± 14[†b]	52 ± 14[†b]
	TS	94 ± 6[a]	92 ± 6[a]	91 ± 6[a]	84 ± 13[a]
	SS	80 ± 11[b]	79 ± 11[a]	81 ± 12[a]	73 ± 15[a]
Carpal	Control	125 ± 3			
	FS	30 ± 9[†b]	26 ± 10[†b]	24 ± 9[†b]	23 ± 9[†b]
	TS*	115 ± 8[a]	114 ± 7[a]	113 ± 8[a]	114 ± 8[a]
	SS*	116 ± 3[a]	112 ± 8[†a]	113 ± 8[a]	119 ± 13[a]
Hip	Control	76 ± 4			
	FS	11 ± 6[†b]	9 ± 7[†b]	9 ± 7[†b]	9 ± 7[†b]
	TS*	71 ± 4[a]	73 ± 6[a]	73 ± 6[a]	74 ± 8[a]
	SS*	67 ± 7[†a]	68 ± 7[a]	67 ± 7[a]	70 ± 5[a]
Stifle	Control	91 ± 6			
	FS	25 ± 11[†b]	20 ± 13[†b]	20 ± 11[†b]	20 ± 9[†b]
	TS*	83 ± 14[a]	85 ± 14[a]	84 ± 12[a]	79 ± 14[a]
	SS*	81 ± 9[a]	76 ± 13[a]	78 ± 12[a]	71 ± 13[†a]
Tarsal	Control	94 ± 4			
	FS	34 ± 9[†b]	16 ± 13[†b]	16 ± 11[†b]	16 ± 12[†b]
	TS*	86 ± 7[a]	86 ± 5[a]	85 ± 5[a]	90 ± 6[a]
	SS*	82 ± 7[†a]	85 ± 7[a]	**85 ± 7[a]**	77 ± 14[†a]

FS = formalin solution; TS = Thiel's solution; SS = saturated salt solution;
P values of < 0.05 indicated statistically significant.
[abc] values with different letters in each month of difference between extension and flexion of range of motion of joints indicate significantly different (*P* < 0.05).
[*] There were no significant difference in TS and SS on every month (*P* > 0.05).
[†] indicates significantly different compared to control group (*P* < 0.05).

Figure 2 **Gross appearance of liver at 2nd months evaluation.** (A) Formalin embalmed liver (B) Thiel's solution embalmed liver (C) Saturated salt solution embalmed liver

Table 4 Color measurements values of color space in biceps femoris muscle and liver of control and three embalming solution groups (mean ± SD)

Color	Site	Group	Time (month)			
			1st month	2nd month	3rd month	4th month
L*	Muscle	Control	32.93 ± 0.93			
		FS	37.17 ± 0.2$^{\dagger b}$	37.86 ± 1.15$^{\dagger b}$	37.38 ± 0.45$^{\dagger b}$	46.60 ± 1.71b$^{\dagger b}$
		TS	29.99 ± 2.47^{a}	33.06 ± 2.13^{ab}	31.32 ± 3.04^{a}	39.61 ± 1.88b$^{\dagger a}$
		SS	29.58 ± 0.47^{a}	29.74 ± 2.70^{a}	32.87 ± 0.8^{ab}	38.06 ± 1.02$^{\dagger a}$
	Liver	Control	35.90 ± 2.71			
		FS	30.78 ± 4.21	29.55 ± 0.16	30.29 ± 1.83^{b}	29.78 ± 0.15^{b}
		TS	33.13 ± 2.52	24.64 ± 2.73	17.38 ± 5.00$^{\dagger a}$	21.80 ± 3.58^{a}
		SS	28.63 ± 1.40	23.83 ± 8.74	17.64 ± 3.53$^{\dagger a}$	17.72 ± 3.54^{a}
a*	Muscle	Control	17.01 ± 1.05			
		FS	11.10 ± 0.6$^{\dagger b}$	9.65 ± 0.77$^{\dagger b}$	12.75 ± 0.54$^{\dagger b}$	10.00 ± 2.43†
		TS	16.58 ± 0.73^{a}	15.03 ± 1.64^{a}	13.54 ± 0.38$^{\dagger b}$	12.74 ± 0.22†
		SS	14.98 ± 2.79^{ab}	14.97 ± 1.37^{a}	15.43 ± 0.03^{a}	9.77 ± 1.09†
	Liver	Control	16.87 ± 2.27			
		FS	8.06 ± 0.64$^{\dagger b}$	8.90 ± 1.22$^{\dagger b}$	7.05 ± 0.67$^{\dagger b}$	7.36 ± 0.60†
		TS	13.01 ± 2.15^{a}	13.46 ± 1.39^{a}	12.50 ± 0.53^{a}	7.55 ± 1.89†
		SS	14.40 ± 1.55^{a}	14.17 ± 0.30^{a}	8.70 ± 1.06$^{\dagger b}$	8.60 ±0.87†
b*	Muscle	Control	10.96 ± 3.45			
		FS	6.32 ± 1.21	5.57 ± 0.12$^{\dagger b}$	5.99 ± 1.05$^{\dagger b}$	3.34 ± 2.52†
		TS	9.20 ± 0.61	11.86 ± 1.06^{a}	7.58 ± 0.37^{ab}	5.12 ± 1.06†
		SS	11.63 ± 0.45	10.10 ± 1.42^{a}	8.24 ± 0.40^{a}	7.22 ± 0.66
	Liver	Control	13.71 ± 1.50			
		FS	5.19 ± 0.98$^{\dagger b}$	5.68 ± 1.64$^{\dagger b}$	5.91 ± 1.36$^{\dagger b}$	5.88 ± 1.39$^{\dagger b}$
		TS	13.84 ± 1.46^{a}	10.20 ± 0.97^{a}	8.87 ± 0.31^{ab}	6.47 ± 0.37$^{\dagger b}$
		SS	12.63 ± 0.45^{a}	12.42 ± 1.24^{a}	13.81 ± 4.48^{a}	14.17 ± 4.84^{a}

FS = formalin solution; TS = Thiel's solution; SS = saturated salt solution.
^{abc} values with different letters in each month of color parameters indicate significantly different ($P < 0.05$).
P values of < 0.05 indicated statistically significant.
† indicates significantly different compared to control group ($P < 0.05$).
L* stands for luminosity indicating darkness or lightness, on a 0 - 100 scale from black to white.
a* indicates redness or greenness, positive value meaning redness and negative value meaning greenness.
b* indicates yellowness of positive value and blueness of negative value.

Discussion

An ideal fixative solution should preserve the structures of tissues and organs during long-term storage, prevent desiccation, maintain flexibility, and retain tissue color (Coleman and Kogan, 1998) and life-like conditions required for surgical training. In this study, the life-like conditions of cadavers were objectively evaluated through the measurement of skin elasticity, joint flexibility, color, and the degree of tissue quality.

The ideal alternative to a live body is fresh cadavers. Although the main advantage of fresh cadavers is the well-preserved anatomical structures, they can only be used for a limited period (Blum, 1893). Formaldehyde was invented by August Wilhelm von Hofmann in 1869 and was introduced as a formalin fixative in 1893 for cadaver preservation (Blum, 1893, Hayashi *et al.*, 2016). Formalin has been widely used because of its antiseptic properties, which can prevent the decomposition of cadavers (Brenner, 2014). However, formalin is unable to provide clinically useful tissue quality (Brenner, 2014; Hayashi *et al.*, 2016; Nam *et al.*, 2020). Indeed, formalin embalmed cadavers have disadvantages when used for surgical practice, such as visual changes, haptic differences, discoloration, rigidity, and desiccation (Hammer *et al.*, 2015; Hubbell *et al.*, 2002; Turan *et al.*, 2017). These limitations have promoted research on alternative fixatives, such as TS and SS, which can preserve life-like conditions for surgical practice. Formalin is also a known human carcinogen, and it is known that the lower the amount of formalin in the fixative solution, the lower the toxicity (Eisma *et al.*, 2013). In previous studies, especially those that used TS with a lower formalin concentration, the odor was evaluated to be lower than that of other solutions, which is likely to be less irritating to the upper respiratory tract (Nam *et al.*, 2020).

Various studies have attempted to provide an alternative fixative for cadavers. Recently, surgical training has been conducted using 3D virtual reality (VR) live models (3D Medivision Inc., South Korea) or synthetic surgical models (SynDaver®, Florida, USA). In the case of 3D VR-based training, education can be provided without additional cost or sacrifice of subjects, once the educational materials and equipment are provided (Hölzle *et al.*, 2012; Peuker *et al.*, 2001). Synthetic models have the advantage of permitting surgeons to acquire practical skills while minimizing sacrifices. However, despite advances in technology, tissue characteristics of synthetic models remain different from those of live animals. Crucially, these models are also expensive and difficult to maintain (Read *et al.*, 2016; Xu *et al.*, 2018). Cadaver alternatives are continuously being studied, but training with original tissues remains superior for developing better surgical skills (Lewis *et al.*, 2012).

The most effective way to replace living animals is by using well-embalmed cadavers with life-like conditions for an extended period (Hölzle *et al.*, 2012). By using an embalmed cadaver, steady and meticulous practice is possible regardless of the operation time (Hölzle *et al.*, 2012). This alternative training meets the ethical aspects and are expected to also be available in other fields, including diagnostic imaging, anatomy, and interventional procedures in internal medicine.

Table 5 Surgical suitability score of each surgery of three embalming solution groups (mean ± SD)

		Time (month)			
Surgery	**Group**	**1st month**	**2nd month**	**3rd month**	**4th month**
Tube intubation	FS	1.2 ± 0.4^{c}	1.2 ± 0.4^{b}	1.3 ± 0.5^{c}	1.2 ± 0.4^{c}
	TS	7.6 ± 1.5^{a}	6.2 ± 1.4^{a}	4.9 ± 0.8^{b}	4.3 ± 0.7^{b}
	SS	6.0 ± 0.7^{b}	6.0 ± 1.1^{a}	5.9 ± 1.1^{a}	5.9 ± 1.1^{a}
Skin flap surgery	FS	1.7 ± 0.9^{c}	1.4 ± 0.7^{b}	1.6 ± 0.7^{b}	1.4 ± 0.5^{c}
	TS	7.6 ± 1.5^{a}	6.4 ± 1.0^{a}	5.2 ± 1.4^{a}	4.0 ± 1.0^{b}
	SS	6.3 ± 1.2^{b}	6.4 ± 0.9^{a}	5.9 ± 1.2^{a}	5.3 ± 0.9^{a}
Lateral ear canal resection	FS	1.7 ± 0.9^{c}	1.4 ± 0.5^{b}	1.4 ± 0.5^{c}	1.3 ± 0.5^{b}
	TS	7.3 ± 1.9^{a}	6.6 ± 1.4^{a}	5.1 ± 1.3^{ab}	4.7 ± 1.0^{a}
	SS	5.4 ± 1.0^{b}	5.7 ± 1.1^{a}	5.7 ± 1.1^{a}	5.3 ± 0.7^{a}
Cystotomy	FS	1.9 ± 1.1	1.9 ± 1.1	1.7 ± 0.9^{ab}	1.4 ± 0.7^{ab}
	TS	2.7 ± 0.7	2.3 ± 0.9	2.0 ± 0.9^{b}	1.9 ± 0.6^{b}
	SS	2.9 ± 0.9	2.8 ± 0.8	2.8 ± 0.8^{a}	2.6 ± 0.9^{a}
Intestinal resection	FS	1.7 ± 0.9^{b}	1.3 ± 0.5^{b}	1.2 ± 0.4^{b}	1.2 ± 0.4^{b}
	TS	7.3 ± 0.7^{a}	7.0 ± 0.7^{a}	5.4 ± 1.0^{a}	4.8 ± 0.7^{b}
	SS	7.2 ± 1.1^{a}	7.0 ± 0.9^{a}	6.2 ± 0.8^{a}	5.7 ± 1.0^{a}

FS = formalin solution; TS = Thiel's solution; SS = saturated salt solution.
^{abc} values with different letters in each month of surgery indicate significantly different (P < 0.05).

Each embalming method has specific advantages and disadvantages. TS has the ability to maintain flexible joints and tissues, a life-like color, consistency, and transparency of tissue, better haptic properties, and proper imaging quality (Eisma *et al.*, 2011; Hammer *et al.*, 2015; Hubbel *et al.*, 2002; Ling *et al.*, 2016). However, TS also has disadvantages, such as deterioration of the cadaver throughout the usage period, inappropriate histological quality, effort and time required for the embalming process, and high costs (Eisma *et al.*, 2011; Hammer *et al.*, 2015; Hubbel *et al.*, 2002; Ling *et al.*, 2016).

Although SS has been studied less extensively than TS, advantages, such as natural color, comparatively lesser deterioration throughout the usage period, good imaging and histological quality, and long-term storage, have been reported (Gosomji 2018; Hayashi *et al.* 2016; Lombardero *et al.*, 2017). However, SS also has disadvantages, such as rigid joints and tissues, edematous changes particularly in subcutaneous tissue, and a change in state during storage (Gosomji 2018; Hayashi *et al.* 2016; Ling *et al.*, 2016; Lombardero *et al.*, 2017). When using life-like cadavers, there are advantages, such as high-quality practice for delicate surgery and repetitive practice opportunities with minimum sacrifice.

Typically, surgeries begin with a skin incision and end with skin sutures, and skin elasticity is an important factor in retracting the skin to cover the defects with tension relief during skin flap surgery (MacPhail, 2018). Thus, skin elasticity is essential in surgical training. In this study, FS cadavers lost skin elasticity from the second month, which was presumed to be due to the rigid fixation and skin shrinkage during formalin fixation (Brent *et al.*, 2005). The TS group cadavers showed changes in skin elasticity from the third month, followed by the SS group cadavers, which started to change from the fourth month. On gross evaluation of each cadaver, skin degeneration (discoloration, keratinization, and nodularization) occurred at the third month in the TS group, and in the fourth month in the SS group. Cadavers in the TS and SS groups were found to be more susceptible to bacterial or fungal infections, which caused damage to the elastic fibers and decreased elasticity relative to those in the FS group (Brenner, 2014; Frances and

Robert, 1984; Hayashi *et al*., 2014; Nam *et al*., 2020). The difference in the degeneration of the embalmed cadavers seemed to depend on the antibacterial effect and extent of infection. In previous studies, TS cadavers were reported to be more susceptible to infection than SS cadavers, and in this study, SS-embalmed cadavers were more suitable for long-term preservation than TS-embalmed cadavers (Hayashi *et al*., 2014; Nam *et al*., 2020). A previous study performed bacterial and fungal culture tests of TS-, FS-, and SS-embalmed cadavers and found that more bacteria and fungi were detected in TS and SS cadavers than in the FS cadavers (Nam *et al*., 2020). For long-term preservation and use of cadavers, application of disinfectants seems necessary to control infection. Lombardero reported hair loss of cadavers, and the same phenomenon was also confirmed in this study (Lombardero *et al.,* 2017). Surgical training was performed in the area where hair loss did not occur, and shaving was performed well before the skin surgery to lessen its impact. As a result, the score of skin surgery tended to decrease over time, which seems to be related to the hair-loss phenomenon.

Joint flexibility is considered one of the most important factors for surgeons in positioning the patient, especially in orthopedic surgery. Surgeries begin with proper positioning of patients to efficiently access the desired organs, and flexibility of the cadavers is important for positioning during surgical training (Jaung *et al*., 2011). In addition, ROM is another important factor because one of the purposes of orthopedic surgery is to restore the normal ROM. It has been suggested that flexibility is determined by the ROM of a joint or a series of joints that are influenced by muscles, tendons, ligaments, bones, and bony structures; hence, flexibility can be analyzed by measuring the ROM values (Krivickas, 2006). The flexibility of cadaveric joints is highly related to changes in soft tissues around the bone. Thus, tissue flexibility can be assessed indirectly using the difference in ROM (Jaung *et al*., 2011).

In this study, differences in extension and flexion ROM were minimal in FS cadavers, while differences in the SS and TS cadavers were obvious. Moreover, differences in ROM were the highest in TS cadavers and the lowest in FS cadavers, without much change over four months. One of the properties of FS is rigidity, and joint flexibility was shown to be poor in FS cadavers. Although all the tested embalming solutions seemed to be suitable if the desired practice was to install implants on the bone, TS would be most suitable embalming solution for orthopedic surgeries to access the joints and bones and evaluate the joint to operate pre-, intra-, and postoperatively. In this regard, TS seemed to be most suitable for surgery requiring specific positions, such as the humanoid or the frog-leg position.

Surgeons must be aware of the structure, location, and color of the normal organs prior to surgery. In addition, the identification of abnormalities in the appearance of tissues and organs, such as ulcers or ischemia, through gross evaluation is imperative to surgeons. Therefore, preserving life-like color of organs is helpful to identify anatomical structures and allows surgeons to perform dissections easily. However, in this study, the tissue color of FS cadavers rapidly discolored. The color of the liver is dark brown to red-brown owing to the cytochromes, carbamino-hemoglobin, and bilirubin contained in the liver, and the biceps femoris muscle is red to pink owing to myoglobin, hemoglobin, and cytochromes (Lone *et al*., 2017). Among them, cytochrome and hemoglobin discolors when exposed to air and gets oxidized; hence, the liver and muscle are suitable for examining fixation-mediated changes over time (Piña-Oviedo *et al*., 2017). In this study, TS cadavers had a life-like color for approximately three months, which would enable effective surgical training. Familiarity with normal structures through well-preserved cadavers using TS can be useful in operations, such as exploratory laparotomy, wherein no lesions are specified. The overall muscle and liver color of the TS showed the highest similarities with the control group at the first and second month. However, at the fourth month, most of TS values were significantly different from those of the control, and some values of the SS tended to be similar to those of the control. Based on these results, SS is more suitable for surgical training over a period of three months or longer. Longitudinal discoloration of organs may also be associated with disease, but further research is needed for confirmation. If the similarities between disease-induced and time-based changes are also revealed, a TS-embalmed cadaver would be an excellent practice model to study both normal and abnormal conditions.

TS can be used for tissue surgeries such as oral surgery, implantology, thyroid surgery, and skin flap surgery, and TS was positively evaluated for surgery within the musculoskeletal system (Eisma *et al*., 2011; Eisma *et al*., 2011; Piña-Oviedo *et al*., 2017). However, TS is unsuitable for surgeries involving nerves and vessels, as it leads to difficulties in identifying thoracic and abdominal organs (Eisma *et al*., 2011; Eisma*et al*., 2011; Hölzle *et al*., 2012). In this study, most surgeons evaluated the TS-embalmed cadavers as being highly similar to living animals, and their soft tissue quality was acceptable for surgical training. As a result, TS was shown to be the most suitable or surgical training in both hard and soft tissues, followed by SS, if used before three months. In this study, the suitability of TS for surgical training seemed to be influenced by various other indicators besides skin elasticity, joint flexibility, and color. However, the SS group showed a better overall score than the TS group at the fourth month, which is presumed to be due to the previously evaluated skin elasticity and discoloration. Unlike other surgical evaluations, TS and SS had poor cystotomy scores, similar to FS. The bladder is normally filled with urine and is a swollen organ. However, during euthanasia, the sphincter of the urethra relaxes and releases urine in the bladder. As a result of being emptied, the appearance and texture of the bladder changes, which affects surgical conditions. Disadvantages due to limitations of the cadaver itself, such as lack of fluidity, pulse, resilience, bleeding, and edema in the event of tissue damage, were noted in the postoperative discussion among the surgeons.

The significant disadvantages of TS embalming are that it is a complicated and time-consuming procedure with a high cost (Lone *et al*., 2017; Macchi *et al*., 2003;

Thiel, 2002; Thiel, 1992). However, once a cadaver is well-preserved, a justifiable calculation of cost is possible by practicing several different procedures on one cadaver. In addition to the use of fixatives in this study, research on the surgical application of various other fixatives is warranted. Simultaneously, it is necessary to evaluate objective indicators to identify surgical suitability of various embalming solutions. In this study, the sample size was small because of limitations of cost, ethical concerns, and research period. Further assessments of various other dog breeds and other organs are desirable.

This study evaluated two alternatives to conventional formalin fixatives. The results demonstrated that TS is the most effective embalming solution for surgical training when evaluated in terms of elasticity, color retention, ROM, and surgical aspects. However, the use of TS for surgical practice seems suitable only if the cadaver is used within three months. Thus, for long-term storage of cadavers, the use of SS is recommended. Effective education based on life-like cadavers can be conducted through a surgical course or wet-lab experience using TS-embalmed cadavers. Importantly, this study is clinically relevant as it suggests the capability of minimizing indispensable animal sacrifice through effective and long-term conservation of quality cadavers.

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