

The Use of Alkyd Resin Method in Wistar Rats for the Preparation of Teaching Materials and Museum Exhibits ^[1]

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^[1] This study was supported by Scientific and Technological Research Council of Turkey [TUBITAK] (Project No: 215O580)

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Article ID: KVFD-2019-23643 Received: 25.11.2019 Accepted: 15.04.2020 Published Online: 17.04.2020

How to Cite This Article

Çınaroğlu S, Keleş H: The use of Alkyd Resin method in wistar rats for the preparation of teaching materials and museum exhibits. *Kafkas Univ Vet Fak Derg*, 26 (4): 507-513, 2020. DOI: 10.9775/kvfd.2019.23643

Abstract

Alkyd Resin method, patented by the Turkish Patent Institute, is a cadaver preparation and preservation technique. This study examines whether exhibition-museum materials can be produced from rat cadavers processed with alkyd resin method in different postures and whether dissection and suturing can be performed on the samples. Besides, samples prepared with alkyd resin and samples prepared freshly were compared in terms of dissection and suturing. 14 Wistar Albino Rats were used in the study. Seven were prepared with alkyd resin method and the others were freshly prepared for comparison in terms of suturing and dissection. The alkyd resin method was applied to two cadavers as a pretrial and to five cadavers for them to be museum-exhibition and educational materials. Alopecia was detected in two cadavers. In some of the exhibition samples processed with the method mentioned, shrinkage and hardening of the skin was noticed. All the samples prepared with this method became products suitable for suturing and dissection. The exhibition-museum materials prepared with the alkyd resin method have maintained their first body postures. We believe that alkyd resin method can be applied to the entire body, and the products can be used for exhibition and educational purposes. Furthermore, the method should be further popularized, and it should be applied to cadavers of different species so that its applicability increases.

Keywords: Alkyd Resin, Cadaver, Museum-exhibits, Rat

Alkid Resin Metoduyla Hazırlanan Wistar Ratlarının Müze-Sergi ve Eğitim Materyali Olarak Kullanımı

Öz

Türk Patent Enstitüsü tarafından patentlendirilen Alkid Resin metodu, kadavra hazırlama ve muhafaza tekniğidir. Bu çalışmada Alkid resin yöntemi ile işlenen rat kadvralarından farklı postürlerde sergi-müze materyalleri üretilip üretilmeyeceği ve elde edilen örneklerin üzerinde diseksiyon ve dikiş uygulamalarının yapılabileceği araştırılmıştır. Buna ilaveten, alkid resinle hazırlanan örnekler ile taze olarak hazırlanan örnekler diseksiyon ve dikiş uygulamaları yönünden karşılaştırılmıştır. Çalışmada 14 adet Wistar Albino Rat kullanıldı. Bunlardan 7 tanesi alkid resin yöntemi ile geriye kalan 7 tanesi ise dikiş uygulaması ve diseksiyon açısından Alkid resinle hazırlanan örneklerle karşılaştırmak amacıyla taze olarak hazırlandı. Alkid resin yöntemi iki rat kadvrasına ön deneme amacıyla beş rata ise müze-sergi ve eğitim materyali olacak şekilde uygulandı. Deneme amacıyla işlenen 2 adet rat kadvrasında kıllarda dökülme belirlendi. Derisi üzerinde adı geçen yöntemle işlenen sergi örneklerinin bazılarında deride büzüşme ve sertleşme dikkati çekti. Bu yöntem ile hazırlanan tüm örnekler dikiş atmaya elverişli ve diseksiyona imkân tanıyan ürünlere dönüştü. Alkid resin metodu ile hazırlanan sergi-müze materyallerinin işlendiği günden beri verilen vücut pozisyonlarını koruduğu gözlemlendi. Alkid resin tekniğinin deri ve iç organlar dâhil tüm vücuda uygulanabildiği, elde edilen ürünlerin sergi ve eğitim amaçlı kullanılabileceği sonucuna varılmıştır. Ayrıca, adı geçen tekniğin yaygınlaştırılması ve uygulanabilirliğinin artırılması için insan dâhil değişik canlı türlerinin kadvralarında çalışılması gerekmektedir.

Anahtar sözcükler: Alkid resin, Kadavra, Müze-sergi, Rat

INTRODUCTION

Anatomy is one of the oldest areas of medicine and one of the cornerstones of medical education. In addition to

providing information to students and physicians, anatomy also designs emotions and thoughts regarding the dead body ^[1,2]. Cadavers have been used for anatomy education and training for centuries. Cadaveric dissection does not only



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provide students with information about the shape and size of organs, but also gives an idea about how each organ is positioned relative to the rest of the body. Dissection is believed to support self-learning and teamwork^[3]. However, in recent years, anatomy education has changed drastically due to financial and ethical concerns, developing technology and difficulties in procuring cadavers^[4]. The importance of anatomy education in medical schools and concerns about teaching standards should be discussed persistently. After many years, Warner and Rizzolo^[5] and Turney^[6] brought forward how anatomy education has fallen below the sufficient level. The way to cope with modern practices is to strengthen the traditional dissection education by introducing innovations into it. Many institutions officially reported that traditional cadaver dissection, which replaced anatomical models and technology, is obsolete^[3,7,8].

The contribution of different methods to education and training in medical schools has always been a research topic^[9]. In schools, small animals such as rats and frogs were often used to teach simple aspects of anatomy. Due to the increasing recognition of animal rights laws, this practice has also decreased in recent years. This has led to the teaching of anatomy in a variety of ways, including Procedures, Problem Based Learning Scenarios (PBL) or more recently computer systems derived from imaging techniques^[3]. In anatomy education, discussions arose about using models, videos, 3D reconstruction and technological devices instead of using cadavers^[4]. However, the studies conducted increase the opinion that education with cadaver facilitates learning, increases respect for body, and positively affects sense of touch and feeling^[10,11]. Animal models and human bodies are utilized in training with cadaver^[12].

Today, studies for education, research and surgical experiences on laboratory animals have gained speed. The cadavers prepared for training are preserved with various solutions or alternative methods. Many of these methods offer excellent opportunities to learn tissues and organs^[3,13].

The most up-to-date technique of preparing cadavers for education and exhibition is plastination developed by Gunther Von Hagens who preserved cadavers by keeping them in different positions^[1]. Developed in the last 30 to 40 years, plastination is a method that allows cadavers to be used in education and training for a long time and that brings a new perspective to gross anatomy^[14]. In particular, with the introduction of plastination technology, the limits of human anatomy samples available for teaching have begun to expand and the potential value of these samples in research has increased appreciably^[15].

Plastinated body or organs do not emit fluids or odors and do not contain insects and bacteria. They do not need extra space for storage, do not require maintenance and are harmless^[16].

As it was reported by the visitors, compared to the odor, wetness and disturbing appearance in human and animal cadavers prepared using traditional methods, there was no odor, wetness and disturbing appearances in bodies created using this method, which was displayed in museums with the name "Body Worlds" in many countries (Japan, Germany, Belgium, England and the USA) and attracted considerable attention of the participants^[17].

Arı and Çınaroğlu^[18] developed a cadaver preparation method where alkyd resin is used. This method includes various steps. Firstly, in the fixation step, muscles and tissues are embedded in formaldehyde solution. Secondly, in the dehydration step, tissues are dehydrated and de-fatted. The third step, which is the embedding step, reduces the volumetric loss and softens the cadaver so that surgical interventions on the cadaver can be performed. In the fourth step, the pre-drying step, the excess chemical is drained out. The fifth step, impregnation step, allows the tissues to retain their peculiar properties after the specimen is treated with a preservative solution containing Alkyd resin and a solution containing toluene and xylene. Finally, the post-drying step is the step when alkyd resin in the specimens are hardened after being dried. Since alkyd resin is a natural resin, cadavers prepared with this method provide a great advantage since they do not require any special storage-preservation conditions in addition to having normal color and consistency being elastic and odorless so that intervention can be performed on them^[19-22].

Working with laboratory animals requires staff who is experienced, trained and knowledgeable in biosafety regulations. Well-trained and experienced staff is very important practically, theoretically and also in terms of implementing international ethical regulations (Three R's principle of Russell and Burch)^[13,23,24].

In this study, we aimed to examine the applicability of the Alkyd resin method to the whole body, skinless or with skin, its usability in the preparation of educational and museum-exhibition materials, and whether it will allow suturing and dissection on the produced materials.

MATERIAL and METHODS

The study was approved by the Animal Experiments Local Ethics Committee of Van Yüzüncü Yıl University (Decision no: 275521-238 Date: 11.05.2015).

In the study, the use of living material and texture was planned in two different ways.

1. In the experimental animal unit, of the 14 adult *Wistar Albino Rats* (11 males, 3 females) weighing 250-350 g, which were excluded from production and intended for sacrifice at various times, seven of them were prepared with the alkyd resin method to be used as sample and seven of them (whole body cadaver, including internal organs) were freshly

prepared for comparison with those prepared with alkyd resin. The combination of 5-8 + 75-90 mg/kg IM Xylazine + Ketamine was used for euthanasia. The abdominal aorta of the animals deeply euthanized were cut, and their blood was drained out.

2. In the study titled "Examination of the effects of selenium in protecting against the negative effects of amiodarone on the thyroid" conducted in the experimental animals unit, some of the body parts of the animals taken as control group were kept fresh for use in the panel, while others were prepared with alkyd resin in addition to the seven whole body cadavers again for comparison on the panel.

Comparison Panel

Seven whole body cadavers prepared with alkyd resin, seven fresh whole body cadavers taken out of cold storage and body parts were taken to the room where the panel would be held to perform the dissection and suture processes. The panel was held with a total 35 participants in total consisting of lecturers and instructors (PhD students) in the medical and veterinary faculties (eight surgeons, two of whom were general surgeons, seven anatomists, four pharmacologists, four zootechnicians, two histologists and three biochemists who did not know how to perform suture, three physiologists, two internal diseases specialists and two gynecologists). However, five people who did not know how to perform suture were removed from the evaluation. Suture was applied to cadavers' muscle tissue in the form of simple suture. The people participating in the panel were taken to the hall where the panel was held one by one and asked to answer the questions given in *Table 1*. The answers were analyzed with the Mann-Whitney U Test.

Preparation of Fresh Cadavers

In the aforementioned study, the sacrificed body parts of the rats used as the control group were kept in the cold storage at -18°C until the day that the panel was held.

Application of the Alkyd Resin Method

Two of the seven whole body cadavers were processed with

the alkyd resin method for trial purposes (the application of the method by determining the waiting period in solutions). According to the trial results, five rats in different postures were prepared with the same method to be used as educational and exhibition-museum material. Fixation, washing, dehydration, embedding, pre-drying, impregnation and final post-drying procedures were applied to each sample prepared with the alkyd resin method.

Fixation

The first step of the alkyd resin method is fixation. A catheter was placed into the abdominal aorta of the materials to be prepared for demonstration. After the probing, the vessels were washed with the physiological saline solution administered. Then, the cadavers were brought to the desired position with the help of various ropes, fishing line, cloth and a small hanging apparatus (*Fig. 1*). Following the position procedure, the animals were fixed by administering Spence's cadaver fixing fluid via the same vessel by means of the catheter. For a 64 kg living being, this fixing fluid is obtained by mixing 2 L formaldehyde, 4 L methyl alcohol, 600 mL glycerin, 800 g phenol and 3 L water^[25]. The materials, which were applied the fixing fluid were placed in 10% formalin, and the fixing process continued for one month. Before proceeding from this step to the next, the cadavers were properly dissected.

Washing

Samples taken from the fixation were washed with tap water for 24 h, and the excess formaldehyde was rinsed out.

Dehydration

The washed samples were processed through 50%, 60%, 70%, 80%, 90% and 96% alcohol series. The samples were kept for approximately eight hours in each alcohol series. The samples taken out of the alcohol were then kept in alcohol-acetone (50% alcohol and 50% acetone) solution for 24 h. In the last part of the dehydration step, the materials were immersed in acetone at room temperature

Table 1. Panel questions asked for materials prepared fresh and with alkyd resin

Applicability characteristics: Dissection and incision
√ Dissection and incision are not applicable (0-2)
√ Dissection and incision are very difficult (3-5)
√ Dissection and incision provide limited opportunity (6-8)
√ Dissection and incision are applicable (9-10)
Do you know how to perform suture?
Yes √ No √
If your answer is yes, please mark one of the following
√ Cannot be sutured (0-2)
√ Very difficult to suture (3-5)
√ Can be sutured (6-8)
√ Can be sutured very easily (9-10)



Fig 1. Rat cadaver, which was administered the fixation procedure

for 48 h. In this way, the fat and water levels in the samples were minimized.

Embedding

Dehydrated samples were placed in glycerol in vacuum desiccators and left under vacuum (220-380 mm Hg) for three days. Thus, the tissues hardened by acetone were partially softened.

Pre-drying

The samples taken out of glycerol were kept on a blotting paper for three days and cleared of excess glycerol.

Impregnation

Pre-dried materials were then immersed in alkyd resin solution in containers with a vacuum feature. They were kept in the vacuum (220-380 mm Hg) for 48 h. Thus, the alkyd resin was ensured to penetrate into the materials, and the impregnation phase ended.

Post-drying

The materials that thoroughly absorbed the alkyd resin solution were left to dry under normal room conditions for 15 days, and the alkyd resin method was finalized [18,19,21]. All the products were then taken onto the exhibition stand to protect them from damage after use.

RESULTS

The most challenging thing in the presented study was keeping the cadavers in the appropriate position while performing fixation. Alopecia, and shrinkage and hardening of the skin were detected in two rat cadavers used in the trial study (Fig. 2). It is believed that the shrinkage and hardening of the skin, and alopecia are related to the waiting periods during the preparation of the materials. The preparation of the other five cadavers took 26 days, excluding the fixation period. The educational and exhibition-museum materials prepared with the alkyd resin method were found to have preserved their positions since the day they were processed. The original structure and size of the cadavers abdominal and chest organs were also observed to have been preserved (Fig. 3, 4). The panel results for dissection and suturing applications are presented in Table 2. According to the results in Table 2, the difference between fresh and alkyd resin cadavers

was revealed to be statistically insignificant in terms of dissection and suturing applications (Fig. 5). The obtained samples have been preserved under room conditions for 46 months.

In terms of dissection characteristics, it was determined that the group with the alkyd resin behaved like fresh tissue and both groups were easily dissectable according to the participants' responses. Although there was no statistical difference between the responses given to the question regarding the applicability of dissection and incision for both groups, both groups were found to be 100% applicable regarding dissection and incision.

In the study, the participants of the panel stated that cadavers prepared with the alkyd resin behaved like fresh cadavers, and suturing was applicable for both groups.

DISCUSSION

Similar to other studies [18,19,21] conducted using the same alkyd resin method, in the present study, an apparent yellow color was not noticed among the observed colors of the samples obtained with the Alkyd resin method.

According to the anatomical study conducted by Thomas et al. [26], the presentation style of the anatomical study materials and the lack of the interrelationship between tissues and organs (the presentation of organs separately outside the cadaver) can be a disadvantage for training with cadaver. Whole body cadavers were prepared for the first time with this method, and it was concluded



Fig 2. Alopecia and shrinkage of the skin

Processing Properties of Materials	N	Group	Median	M	SD	Min.	Max.	P
Dissection Characteristics	30	Fresh	10.00	9.30	1.70	4.00	10.00	.21
		A. Resin	10.00	9.80	0.76	7.00	10.00	
Can Be Sutured (Yes)	30	Fresh	7.00	7.73	2.34	2.00	10.00	.79
		A. Resin	7.00	8.11	1.49	7.00	10.00	



Fig 3. Products for educational and museum-exhibition purposes prepared with the alkyd resin method

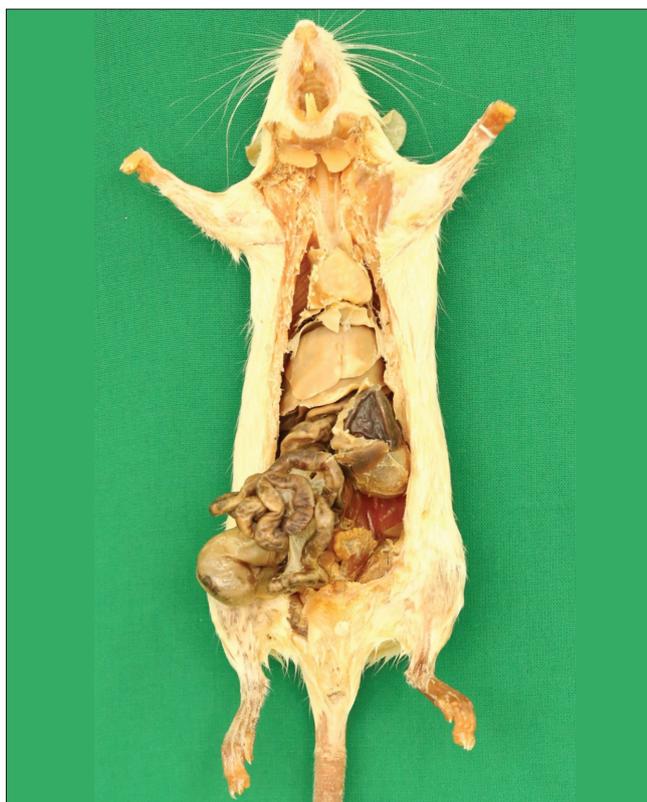


Fig 4. General view of the internal organs in cadavers prepared with the alkyd resin method

that there was no change in the shape and size of the internal organs and the total structure of the cadaver. The cadavers prepared with alkyd resin method in this study, will eliminate the disadvantage of the lack of presentation stated in Thomas et al.^[26] in terms of shape and image.

In their study, using an acetone meter, Ekim et al.^[27] maintained the dehydration phase of the plastination method until the acetone concentration was balanced. In our study, it was revealed that there is more than one phase in the dehydration phase and an acetone meter cannot be used because of the alcohol use in these phases, and that keeping the samples in the dehydration phase longer causes shrinkage and hardening of the skin. However, it was also determined that establishing a standard in the application of this method for processing the skin of all other living groups was necessary.

Panel participants who knew how to perform sutures evaluated the samples prepared fresh and with the alkyd resin as "sutureable". Only one panel participant (3.3%) emphasized that fresh tissues cannot be sutured. 100% of the participants stated that tissues with alkyd resin can be sutured. Panel participants expressed that dissection can be performed in both groups. This result coincides with the study results of Arı and Çınaroğlu^[18], and Çınaroğlu and Arı^[19] who stated that procedures such as dissection and suturing can be performed on tissues with alkyd resin.



Fig 5. Incision and suturing applied materials

Lewis et al.^[28] argued that anatomical knowledge is required to learn surgery, that basic anatomical information and dissection times offered in the first year are decreasing, and that intern students do not remember this basic anatomical information. The authors state that detailed anatomical knowledge yields a high level of tissue and organ manipulation by improving the efficiency and safety of a surgeon in order to heal and save a patient, which is called anatomical engineering. They argue that while anatomy can also be learned from textbooks, atlases, computer models and projections, dissection is the most efficient method. In the same study, it was emphasized that the fact that endoscopic interventions, which largely replaced open surgeries, are performed on a two-dimensional screen limits the examination of the anatomical structures of the three-dimensional organs. Based on these arguments, they concluded that dissectible cadavers prepared fresh or prepared with alternative methods will increase knowledge and skill in surgical education and specific surgeries. There are also some other studies^[29,30] where it is suggested that plastine rodents can be an excellent resource to learn their anatomy and to gain knowledge, and can be useful for innovative applications such as developing practice, comprehension, understanding complex anatomical relationships, surgical maneuvers, endoscopic trials, and developing autopsy protocols^[13]. In addition to all these mentioned elements, it is reported that learning a new procedure with daily practices on living beings is quite dangerous for education and that practices on cadavers increase the education experience^[31].

The present study proved that the alkyd resin method can be applied to the whole body and supported the arguments of Huri et al.^[12], Lewis et al.^[28] Stuart and Henry^[29], Latorre et al.^[30] and Tjalma et al.^[31]. They argued that education with cadavers should not only be offered during schooling but should also be used in surgical training, the teaching of various surgical interventions, trial of new procedures and training of specialist physicians.

According to a study, it is estimated that 192.1 million experimental animals (including those that were produced for scientific studies but were not used or could not be used) were used worldwide in 2015^[32]. According to another study, it is stated that approximately one third of the experimental animals are lost as a result of education and wrong practices^[33]. People working on laboratory animals should complete their education by avoiding toxic effects of the samples reflecting real tissue and by using the least number of animals^[34]. Working with harmless materials reflecting the real tissue also reduces the anxiety that trainees and scientific researchers may have while handling live animals^[34]. Within the framework of the 3R rule and due to ethical concerns, the alkyd resin method may be useful in order to prevent or reduce this number of animal losses in animal experiments. We produced rat cadavers that can be preserved in room conditions for 46

months. In studies conducted with lab animals, specifically with rats, these cadavers can be used for determining the places of organs and tissues, recognizing normal sites, marking the surgical intervention points, giving training before the study, teaching about anatomic structures, and preventing concerns that may occur with live animals.

With the innovations it will bring to anatomy education, the alkyd resin method may be an alternative to the old dissection methods mentioned by McLachlan et al.^[3], Guttman et al.^[7] and McLachlan^[8]. On this subject, the studies conducted by Arı and Çınaroğlu^[18], Çınaroğlu and Arı^[19], Çınaroğlu et al.^[20], Keleş and Çınaroğlu^[21], and Çınaroğlu and Keleş^[22] contribute to gross anatomy. Similar to the abovementioned studies, this study also developed inexpensive anatomical models that can be preserved in room conditions for a long time. In addition to the mentioned studies, this study proved that these anatomical models can be dissected and incised and are suitable for suturing. Furthermore, with the six exhibitions opened in various regions in Turkey so far, this method is estimated to be a method that can be used together with the plastination method. These rat cadavers, which have been produced so far, have been presented to the scientific world for the purpose of exhibition and education, and academicians who want to conduct scientific studies and receive training visit the hall where cadavers are exhibited. In addition to the advantages brought by plastination of rodents stated in Ottone et al.^[13], Stuart and Henry^[29] and Latorre et al.^[30], the alkyd resin method can offer similar advantages in anatomy classes and postgraduate studies.

In conclusion, given the purposes of the study, the alkyd resin method can be applied to the entire body, including skin and internal organs, and the products obtained can be used for exhibition and educational purposes. The technique in question should be applied to cadavers of different species to pursue its dissemination and to increase its applicability. Furthermore, it is believed that the examination of the alkyd resin method's dyeability characteristics like the ones in the plastination method, which we consider as a reference method in preparation of museum and exhibition materials is necessary.

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