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PRESERVATION OF CANINE FRONT AND HIND LIMB SPECIMENS USING PLASTINATION TECHNIQUE: IMPREGNATION PHASE IN A PASSIVE VACUUM CHAMBER

Pengawetan Spesimen Kaki Depan dan Kaki Belakang Anjing Menggunakan Teknik Plastinasi dengan Fase Impregnasi dalam Ruang Vakum Pasif

Luh Gde Sri Surya Heryani*, I Nengah Wandia, I Ketut Suatha, Ni Nyoman Werdi Susari

Laboratorium Anatomi dan Embriologi Veteriner, Fakultas Kedokteran Hewan, Universitas Udayana, Kampus Bukit Jimbaran, Badung, Bali, 80361, Indonesia;

*Corresponding author email: surya_heryani@unud.ac.id

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Abstract

The plastination technique is a preservation method that involves the infusion of polymer materials into biological specimens to maintain their structural integrity, prevent decay, and ensure long-term durability. This technique is widely regarded as an effective approach for preserving organs and is particularly valuable for anatomical education. The objective of this study is to evaluate the qualitative characteristics—specifically texture, color, and odor—of plastinated canine front and hind limb specimens during the impregnation phase, conducted within a passive vacuum chamber. Additionally, this research aims to propose recommendations for refining plastination techniques to enhance the quality of preserved organ specimens. The study employs a custom-designed apparatus and readily available generic chemicals to perform the plastination process. The plastination procedure consists of four key stages, with the impregnation phase being carried out in a vacuum chamber utilizing a passive vacuum system. The resulting plastinated specimens were assessed for flexibility, color, and odor. The findings revealed that the plastinated organs exhibited a rigid texture, a pale coloration, and a mild odor. The anticipated outcome of this research is to provide actionable recommendations for improving plastination methods, thereby enhancing the quality of plastinated organ products for use in veterinary anatomy education.

Keywords: plastination technique, front limbs, hind limbs, impregnation phase, vacuum chamber

Abstrak

Teknik plastinasi merupakan proses pengawetan organ dengan memasukkan bahan polimer untuk mempertahankan bentuk dan komposisi organ, dengan tujuan mempertahankan keutuhan organ dalam waktu yang lama dengan cara mencegah kerusakan dan proses pembusukan lebih lanjut. Organ hasil pengawetan dengan proses plastinasi menjadi pilihan sebagai media

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pembejaran anatomi. Tujuan penelitian ini adalah untuk mengevaluasi kualitas organ plastinasi terutama tekstur, warana, dan bau yang tahap impregnasi pada ruang vakum pasif, serta menyusun rekomendasi remedial teknik plastinasi sehingga menghasilkanproduk organ awetan yang berkualitas. Penelitian ini dirancang untuk mengkaji secara kualitatif produk dari suatu teknik plastinasi yang dilakukan dengan menggunakan bahan kimia generik di masyarakat dan peralatan yang dibuat sendiri. Spesimen yang akan diplastinasi adalah potongan kaki depan dan kaki belakang anjing. Teknik plastinasi melalui empat tahapan utama, salah satunya fase impregnasi yang akan dilakukan ruang vakum dengan mekanisme vakum pasif. Organ plastinasi yang merupakan produk dari teknik ini akan dievaluasi terhadap kelenturan, warna, dan baunya. Penelitian ini menghasilkan organ yang mempunyai tekstur yang kaku, warna kepucatan dan bau yang netral atau tidak menyengat. Hasil penelitian ini diharapkan dapat memberikan rekomendasi remedial teknik plastinasi sehingga menghasilkan produk organ plastinasi yang lebih berkualitas untuk pembelajaran anatomi veteriner.

Kata kunci: teknik plastinasi, kaki depan, kaki belakang, fase impregnasi, ruang vakum

INTRODUCTION

In the world of education, especially studying anatomy, organs or cadavers play a very vital role as a learning medium. To be able to extend the function of organs or cadavers as a learning medium, the organ or cadaver needs to be preserved. The current method of preserving organs or cadavers involves the use of formalin and/or freezing them at a temperature below the water freezing point. The purpose is to maintain the integrity of organs for an extended period by preventing further damage and decay processes (Musiał et al., 2016; Amirudin & Putra, 2023).

Traditional organ or cadaver preservation is relatively straightforward, but it has several disadvantages. Organs or cadavers that have undergone formalinization frequently exhibit rigidity and a potent odor, accompanied by a pale and grayish coloration. Additionally, the organs within the body may undergo displacement. In contrast, the freezing of organs or cadavers can result in increased rigidity, making the learning process difficult. Frozen organs or cadavers need to be thawed first before use. The process of thowing has the potential to induce the decay and subsequent destruction of the organ or cadaver, thereby reducing its longevity (Musiał et al., 2016; Amirudin & Putra, 2023).

Learning using organs and cadaver dummies does not adhere to the minimum criteria for anatomy learning media as it fails to accurately replicate the physiological conditions of the human or animal body. One of the most suitable methods for studying anatomy is through the utilization of preserved organs or cadavers. The process of preserving organs through the use of formalin results in various physiological effects, including increased rigidity, a strong odor, a slight alteration in color to a pale and grayish color, and potentially even a displacement of the organ within the body (Musiał et al., 2016).

Plastination is an anatomical technique used to preserve biological material with educational and training purposes. It was developed by Professor Gunther von Hagens in Heidelberg, Germany, in 1977, as a way to preserve human organs or cadavers. This preservation of human organs and cadavers was intended to create a medium for medical students to study human anatomy, serving as a substitute for cadavers preserved with formalin. In 1982, plastinated specimens were used for anatomy studies for the first time at the University of Heidelberg, where he worked (Ottone et al., 2014; Ottone et al., 2015). Since then, the use of plastinated organs for studying anatomy at various universities has spread widely, such as Singapore's Nanyang Technological University.

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The organ/cadaver plastination technique is a long process that involves multiple stages. One of the phases involves the dehydration procedure, which is designed to eliminate all water from the organs, tissues, and cells and substitute it with alternative fluids, such as acetone, in order to preserve their structural integrity. The acetone dehydration process is typically conducted at low temperatures (-20^{o}C), which requires a dedicated room or freezer. This can pose challenges when performing organ plastination in an unequipped facility or laboratory. Because of this, the goal of this study was to start using an organ plastination method where the dehydration step can be done at room temperature instead of in a freezer-like cold room, starting in 2013 (Hadi, 2013). It seems that Indonesian universities offering medical, or nursing education do not utilize plastinated organs or cadavers for learning anatomy for their students.

There are multiple phases or stages involved in the organ/cadaver plastination technique. One of the sequential steps involves the impregnation phase, which serves the purpose of eliminating acetone from the organs, tissues, and cells and substituting it with polymer material (specifically silicon) in order to preserve its structural integrity. The impregnation process takes place in a vacuum chamber to facilitate the evaporation of acetone and induce the migration of the polymer into the phase. In this study, the investigators construct a vacuum chamber to reduce costs, and passive impregnation will be conducted in that chamber.

RESEARCH METHODS

Research object

The canine front and hind limbs are the specimens or objects used for the plastination process. The materials include distilled water, formaldehyde, acetone, liquid silicone rubber, and silicon catalyst. The materials utilized in this study will consist of generic materials that are readily available within the community and purchased through either local markets or chemical shops, thereby resulting in cost savings. The research utilized a 10 ml syringe, a lidded plastic bucket/tub, a vacuum chamber, a lidless plastic bucket/tub, and a hardening chamber/box. The vacuum chamber is constructed using a modified drum to accommodate the plastination of large-sized cadavers. The vacuum chamber has been specifically designed to minimize expenses and can be utilized continuously. This plastination technique is designed to eliminate the need for a freezer, resulting in reduced costs for organ plastination in the future.

Research design

This research was carried out using a trial method of four plastination phases: fixation, dehydration, impregnation, cleaning and positioning, and hardening. The process of organ plastination experiments can be shown in Figure 1.

Research variable

Research variables include assessment of plastinated organ texture, color, and odor. Plastinated organs were categorized into three types based on their texture: flexible, moderately flexible, and rigid. The coloration of the plastinated organs was classified into three distinct categories: fresh, pale, and grayish, while the odor was categorized into levels of neutrality, mild pungency, and strong pungency.

The organ's texture, which is formed through plastination, is considered flexible if it can revert back to its initial position within a maximum of 5 seconds when subjected to pressure. The organ is moderately flexible if it reverts back to its initial position within a time frame of 6 to 15 seconds. Meanwhile, a rigid organ is characterized by the inability to bend or revert to its initial position within a time frame exceeding 15 seconds.

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A plastinated organ is considered fresh if its color is vibrant, resembling that of a fresh organ. The organ is considered pale when the color fades slightly and grayish when the color is gray. The odor of plastinated organs is considered neutral if it does not cause nasal irritation. The organ is classified as mildly pungent if it elicits mild nasal irritation upon inhalation, whereas it is strongly pungent if it causes irritation to both the nose and eyes.

Data analysis

The plastinated organs will undergo a qualitative analysis. The results of this evaluation will serve as a reference for subsequent improvements in plastination techniques.

RESULTS AND DISCUSSION

Results

The canine front and hind limbs have been preserved through a plastination technique that involves an impregnation phase in a passive vacuum chamber. This procedure has been conducted to obtain organ cadavers that can be utilized for educational purposes in the veterinary anatomy laboratory. The front limb specimen plastination process consists of the following phases: fixation, dehydration and defatization, impregnation, cleaning and positioning, and hardening (Figure 1).

Discussion

Anatomy serves as the fundamental basis for the majority of health professions, with the human body being the preferred instructional tool for understanding the diverse anatomical structures of the human body. The feasibility and efficacy of cadaver preservation have facilitated its application in educational contexts (Balta et al., 2015).

Organ preservation is the process of maintaining the integrity of organs for a long time, which can prevent further decay. The current methods for organ preservation encompass formalin, freezing, and plastination techniques (Fruhstorfer et al., 2011). Each method has advantages and disadvantages. The formalin preservation method produces organs and cadavers that have a rigid texture, a pale/grayish color, and a strong odor. The advantage is that it can be used for a long time. The freezing preservation method results in organs/cadavers with a rigid consistency, which requires a long process to restore them to their initial condition/consistency. The benefit lies in its long-term usability. Plastination is a technique that yields organs or cadavers that closely resemble their original form, are odorless, but require a long procedure and costly equipment and materials.

Anatomical specimens that are non-toxic and suitable for long-term educational use are created through the process of plastination, which involves preserving tissue, organs, and the entire body. Dr. Gunther von Hagens was the one who first created it in 1977. The controversial traveling exhibition "Body Worlds" helped this German anatomist, also known as "Doctor Death," gain international recognition for his work (Riederer, 2014).

The notion that plastic polymers can take the place of biological fluids in specific specimens is one of the fundamental concepts behind plastination. The presence of water and fat in biological tissue can be replaced by using certain plastics, such as silicone, epoxy, and polyester. This material can create touchable specimens that do not decay or smell, and they can keep the majority of the original sample's characteristics (Riederer, 2014).

When compared to formalin preservation, plastination has the following benefits: (1). Plastinated sample storage is simpler; (2). The specimens obtained from this process are fully preserved and free of strong odors or formalin fumes; (3). Comparatively less costly over a prolonged period of time; (4). The smell of formalin can occasionally make it difficult for some

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students to study, which eventually lowers student interest; (5). It is possible to preserve specimens for up to 40 years; (6). Because all structures are fully preserved in a nearly natural state, plasticination offers features that are comparatively more detailed; (7). Studying topographic anatomy in detail is made much easier with the aid of sheet plastination; (8). Parasites found in meat, such as larvae in rotten meat, can be preserved for demonstration purposes; (9). Fragile tissue samples, such as intracerebral hemorrhage, can be perfectly preserved and made durable for future use (Atwa et al., 2021). Despite this, there is no evidence to suggest that plastination is superior to conventional cadaveric preparation (Chytas et al., 2019). There are drawbacks to plastination as well, including: (1). Deeper anatomical features are difficult to demonstrate on plastinated specimens due to their relative inflexibility; (2). Plastinated specimens are not the best choice for use in clinical practice; (3). Plastination is a time-consuming and delicate technique that requires skilled personnel; (4). Improper handling of the chemicals used in the procedure can be harmful to one's health; and (5). Pathogen exposure is possible, particularly in the early stages of sample processing (Amirudin & Putra, 2023).

The study's texture and flexibility findings revealed the rigid organs. This is because the impregnation process in a vacuum chamber only takes five hours. It is still not enough time to push silicon rubber to replace acetone in the organs. Placing a volatile solvent (acetone, etc.) with a mixture via a silicone reaction is the fundamental idea behind impregnation. A vacuum device is needed so that the thick silicon mixture can reach equilibrium with the dehydration agent. For the thick silicon mixture and the dehydration agent to come into equilibrium, a vacuum device is required. Ideally, forced impregnation is done to prevent specimen shrinkage (Dejong & Henry, 2007). Despite the fact that this study demonstrates that tissue shrinkage does occur even in cases of forced impregnation, this shrinkage can be reduced by applying the proper vacuum pressure (Singh et al., 2015).

Due to the dehydrating properties of acetone, the resulting color is usually pale. The removal of water and fat from the organs takes place during this phase, and then acetone replaces them. The smell of the organs produced is not strong; this is because the fixation process using formalin is carried out at the beginning of the plastination process, and at the end of the process, the smell of formalin is no longer visible. Plastination techniques involve the substitution of water and lipids in biological tissues with polymers such as silicone, epoxy, and polyester. Once the material solidifies, the specimen is odorless, dry, durable, and easy to transport (Sora et al., 2019).

The findings of this research have not yet achieved optimal outcomes. The organ exhibits a rigid texture due to the limited impregnation time in a vacuum chamber, which is only 5 hours. The current duration remains insufficient to facilitate the substitution of acetone with silicone rubber within the tissue. As an effect of acetone's dehydrating properties, a pale color is commonly produced. The odor emanating from the organ is relatively mild, which can be attributed to the absence of formalin immersion.

CONCLUSION AND SUGGESTIONS

Conclusion

According to the research findings, the plastinated organs of the canine front and hind limbs exhibit a rigid texture, a pale color, and a mild odor.

Suggestion

For the resulting plastinated organ to have a texture that more closely resembles its original shape, it must be stored in a vacuum for a longer period of time (one week).

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Tables

Table 1. The definition of each level of variable is:

Levels	Texture	Information
1	Flexible	Return to original position within a maximum of 5 seconds
2	Moderately flexible	Return to original position within 6 – 15 seconds
3	Rigid	Cannot be bent, cannot return to its original position, or returns to its original position in more than 15 seconds
4	Fresh	The color is bright, like fresh organs
5	Pale	The color is a little faded
6	Grayish	The color is gray
7	Neutral	Does not cause nasal irritation
8	Mildly pungent	Causes slight nasal irritation when inhaled
9	Strongly pungent	Irritates the nose and eyes

Table 2. Observation Results of Front and Hind Limb Plastination

	Organ	Quality		
		Texture	Color	Odor
	Front Limbs	Rigid	Pale	Neutral
	Back Limbs	Rigid	Pale	Neutral

Figures

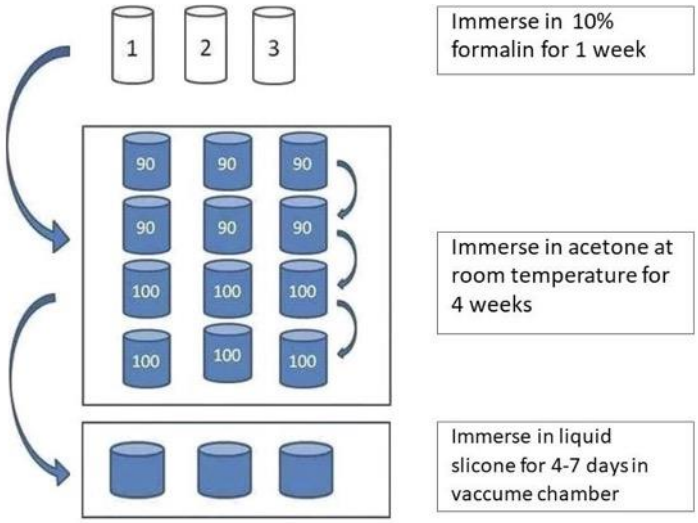

Figure 1. Animal organs plastination process flow

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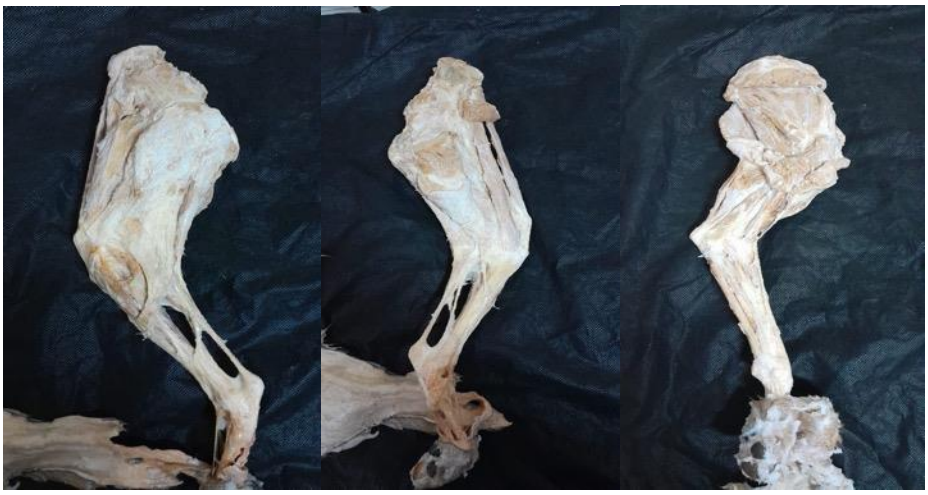

Figure 2. Day 2 of the curing process. The result is that most of the silicon has hardened, the organs are hardened and slightly shriveled, the color is slightly faded from fresh, the smell is normal (not strong). Durability in 2 days remains

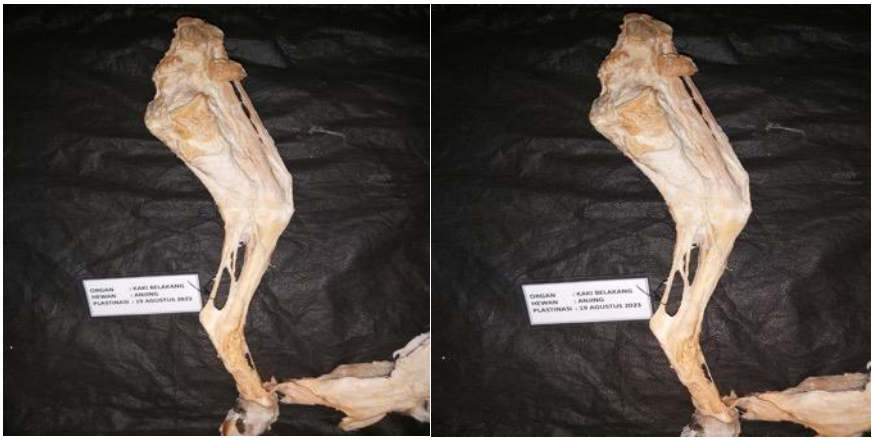

Figure 3. Plastinated canine hind limb 19 August 2023

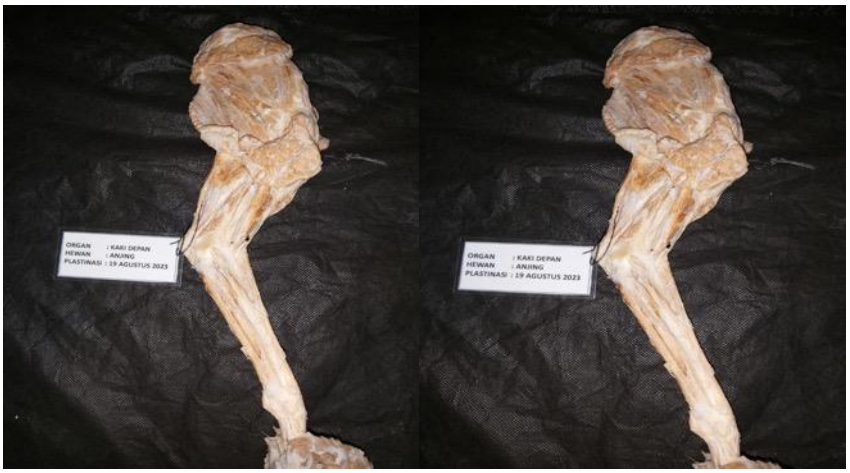

Figure 4. Plastinated canine front limb 19 August 2023

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