Silicone Plastination of Nasus and Lingua in Large Ruminants with and without Use of Formaldehyde

Plastinación de Silicona de Nasus y Lengua en Rumiantes Mayores con y sin el Uso de Formaldehido

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SUMMARY: Plastination is an anatomical preparate preparation technique characterized by the replacement of tissue fluids with a reactive polymer. Although more challenging and economically costly than many anatomical methods, this method is desirable because of the fact that specimens created in this method are highly similar to the natural appearance of the intended objects, and they are durable and harmless end products for human health. Our main goal was to completely leave out formaldehyde and similar carcinogenic chemicals used in a method like plastination and to allow production of formaldehyde-free plastinates to be used in anatomy training and examinations in our country. To that end, we compared nose and tongue of 10 large ruminants by subjecting them to plastination, 5 of them with formaldehyde and 5 of them without formaldehyde, and aimed to leave formaldehyde out by taking into account the difference between them. Silicone plastination is the most commonly-used and best-known technique among the plastination techniques because specimens created using this technique look aesthetically impressive. Silicone plastination consists mainly of 5 phases. First of all, we obtained the anatomical situs we wanted and made specimens ready by dissecting some of them after fixation and some of them without fixation. Then, after the implementation of a dehydration phase in acetone baths at -25 °C, a forced impregnation phase, the plastination process was completed by giving the specimens their final shape with the use of the S6 solution. As a result, no significant difference was observed between silicone plastination with and without formaldehyde.

KEY WORDS: Anatomy; Formaldehyde; Large ruminants; Plastination; Silicone

INTRODUCTION

Cadavers used as learning materials in medical and veterinary education are indispensable for macroscopic anatomy. Although today formalin is used to preserve cadavers, it is clear that it is difficult to work on and have students practice on organs stored using formalin. Formalin in tissues or organs evaporates into the air of the environment and causes skin and eye irritations. If formalin is contacted unprotected, it causes damage to the skin and mucous membranes. This adversely affects the comfort in the education environment, causes tissues or organs to lose their natural appearance, makes it difficult to study them macroscopically by holding them by hand, thus reducing students interest in the course (Gültiken, 2012).

Cadavers are kept in tanks filled with solution created from 5-10 % formaldehyde or its derivatives.

Tissues in cadavers obtained using such formaldehyde based solutions are wet and slippery, making it difficult for users to work on cadavers, and their preservation varies from 1 to 2 years on an average (Alyüz & Veli, 2006).

Plastination was first discovered in 1977 by Dr. Gunter von Hagens at the Institute for Anatomy at Heidelberg University, and the first information about it was published in 1979. In the plastination method described by Dr. Gunther von Hagens, synthetic polymers such as silicone, epoxy and polyester replace water and fats in biological tissues and solidify, and they turn this biological material into a structure that is dry, odorless, durable and perhaps most importantly non-harmful to health, closest to its original appearance (Pashaei, 2010; Singh *et al.*, 2013).

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Plastination can be described in general as a process of removing tissue fluids from tissue by using acetone, alcohol and similar solvents, replacing them with a polymer chemical derived from silicon, polyester or epoxy, and fixing the chemical in tissue (Ekim *et al.*, 2014a).

Plastination by using silicone (S10 technique) is the most common standard technique. S10-impregnated specimens have an opaque, somewhat flexible and natural-looking appearance. This procedure consists of 5 main phases.

These can be summarized as preparation of specimens (fixation/dissection), dehydration, impregnation and gas curing/hardening, in the order given (De Jong & Henry, 2007).

Lingua (tongue) is an organ with a muscular structure. The tongue is responsible for the intake of liquids and nutrients into the mouth, mixing and chewing the liquids and nutrients taken into the mouth, and swallowing them. It is located at the base of the mouth cavity. It extends to oropharynx, covering most of the cavum oris proprium. The tongue consists of the tip (apex linguae), body (corpus linguae) and root (radix linguae) sections (Konig & Liebich, 2015; Dyce *et al.*, 2018).

The skeleton of the nasal cavity (cavum nasi) is shaped by palatine process of incisive bone, palatine process of maxilla and horizontal plate of palatine bone ventrally, maxillary corpus laterally, and nasal bone dorsally. It is surrounded by lamina cribrosa of ethmoidale caudally. It extends to nasopharynx ventrally. Septum nasi divides the nasal cavity into two sections as the right and left ones. It is made of hyaline cartilage (Dyce *et al.*, 2018).

This study was realized to carry out silicone plastination of the nose and tongue of large ruminants at room temperature by handling them primarily in 2 groups (with and without fixation by using formaldehyde). Thus, thanks to the plastinates created, formaldehyde-like carcinogenic fixation solutions will be avoided. Fixation solutions such as formaldehyde and its derivatives are used for fixation purposes even in the plastination method in the first phase. The main objective of this study was to compare the two groups silicone plastination implemented by fixation with and without formaldehyde and examine the differences that occurred. Thus, the aim was to remove formaldehyde completely from our lives and to ensure the spread of plastinates, each of which would be original, produced in our country for use in anatomy training without formaldehyde.

MATERIAL AND METHOD

Ethical approval was obtained from the Presidency of Animal Experiments Local Ethics Board of Firat University with the approval no. 16.11.2016/ 194. A total of 10 large ruminant skulls were obtained from the slaughterhouses of Elazıg' province, Turkey, dissected and their tongues were removed to expose their nasal pathways. Silicone plastination was performed on 5 of them by fixing them with 10 % formalin solution. And the other 5 were subjected to silicon plastination without undergoing any treatment. All of the silicon plastination procedures reported in this study were performed at the Plastination Laboratory of the Department of Anatomy at the Faculty of Veterinary Medicine, Firat University. The terminology of the Nomina Anatomica Veterinaria was considered as the basis (International Committee on Veterinary Gross Anatomical Nomenclature, 2017).

The plastination method consists of five basic steps (Pashaei, 2010; Singh *et al.*, 2013).

1. Preparation of specimens (Dissection/Fixation): The 10 large ruminant skulls obtained from the slaughterhouses of Elazıg province were brought to the desired shape/position for the final presentation and dissected to remove any unnecessary connective and fat tissues. First of all, the tongues were separated from their skulls and made ready. Then, the skulls were longitudinally and transversely cut with the help of a saw. Thus, nasal pathways and conchae were visible in the longitudinal sections. The specimens of the group to be fixed by using formaldehyde that were ready to be processed were held in 10 % formalin solution at room temperature for an average of 3 weeks. Since the specimens we used were solid organs in general, we waited for this duration for our fixation solution to reach from the periphery to the center. The specimens that were not fixed by using formaldehyde were taken to the dehydration phase immediately after the dissection phase.

2. Dehydration: An intermediate chemical, expressly acetone, is needed so that in-tissue and between-tissue fluids can be removed and replaced with silicone. Otherwise, this will not be possible due to the high-density difference between the two fluids.

Acetone evaporates very quickly at room temperature and is a chemical that can cause the tissue it comes into contact with to shrink. Therefore, acetone baths are carried out at -25 $^{\circ}$ C.

Dehydration equipment and chemicals:

- The deep freezer was a domestic deep freezer (Ugur Deep Freezer 620 L). Dehydration was performed at a temperature of -25 °C.
- Stainless steel tanks (Kenatek, Bornova, Izmir, Turkey) with a capacity of 35 L and sealed lids were used.
- Two acetonometers were used: between 0 % and 100 % and between 90 % and 100 %. The acetonometers were calibrated to +20 °C (Kenatek, Bornova, Izmir, Turkey).
- Acetone was poured to be 10 times the specimen volume.

After the specimens were washed in tap water, they were given an anatomical posture and left in 99.5 % acetone. Then, by using an acetonometer, the concentration was measured every day at the same time by taking the amount of acetone required for measurement, after the acetone in the tank held at -25 °C was mixed. An important point here is that when acetone concentration is measured, acetone that is used should be waited for to reach the temperature specified in the acetonometer and should be measured after that. The acetone specimens that were taken were measured at 20 °C, as the acetonometer used in this study was set to the specified temperature. The lid of the steel tank was closed, and the tank was left in the deep freezer at -25 °C. The density was checked using the acetonometer every day at the same time. When the density became stable, the second bathing was started. The baths were repeated until the acetone density inside the specimens exceeded 98 %. When it reached a density of 98 % or more, the dehydration process was terminated.

3. Degreasing: After the dehydration process was completed, the specimens were kept in pure acetone at room temperature for 4–5 days, ensuring the removal of the remaining fat tissue residues between the tissues or on them.

4. Forced impregnation: Forced impregnation equipment and chemicals:

- Vacuum tank with transparent lid [tempered glass or polycarbonate] (Thick enough to withstand one atmospheric pressure) (Kenatek, Bornova, Izmir, Turkey)
- Vacuum pump: An oil-cooled pump with slow pump speed was used (Kenatek, Bornova, Izmir, Turkey)
- Vacuum gauge, tubing and fine adjustment needle-valves
- Digital manometer
- Specimen basket to place the specimens in
- BIODUR \$10-\$3 mixture (at 100:1) (Biodur Products, Heidelberg, Germany).

After the specimens came out of the degreasing phase, they were immediately transferred to the metal basket and placed in the silicone (S10) in the vacuum tank at room temperature (Ottone *et al.*, 2015). The amount of S10 in the tank was topped up to exceed the top of the basket a little bit. S3 catalyst was added on the basis of a ratio of 100:1 to activate the S10 mixture (Ottone *et al.*, 2015). After the specimens were placed in the S10/S3 mixture, weights made of stainless steel in a perforated dissections were carried out on deformed anatomical structures of the specimens during this waiting process. After the silicone release stopped, excess silicone was blotted from the specimens by using dry and lint-free absorbent papers, and the next phase began.

5. Gas curing: Necessary equipment and chemical used for gas curing:

- Air-tight closed container which is large enough to contain curing unit specimens (Kenatek, Bornova, Izmir, Turkey)
- Aquarium pump or small ventilator (fan)
- BIODUR S6 chemical (Biodur Products, Heidelberg, Germany).

In addition to the specimens, S6 was placed in the gas curing tank in a glass container with its lid open. The plastic tubing carrying the air from the aquarium pump was placed in the glass container containing the S6. The aquarium pump was operated for one hour, 2 times per day throughout the curing phase.

After ensuring that the specimens hardened, the specimens were kept in closed containers.

After completion of the dissection procedures in the silicon plastination process performed on the noses and tongues of large ruminants, the 2 groups (including the organs that were fixed and the organs that were not fixed) were taken separately to the dehydration phase. During the dehydration phase, the specimens that were fixed had to be taken into acetone bath at -25 °C twice. The organs that did not undergo fixation were taken to the acetone bath at -25 °C 3 times. The dehydration period of those fixed by using formaldehyde continued for 14 days. The specimens that did not undergo fixation continued for 19 days. Concentration data recorded throughout this phase were shown in Tables I and II. The degreasing phase of the 2 groups, whose dehydration phase was completed, lasted 5 days at room temperature.

During the forced impregnation phase, pressure was observed through bubble output. In the specimens undergoing formalin fixation, the first bubble output began at 640 mmHg, and impregnation took place for 6 days (Table III). In the specimens that did not undergo formalin fixation, the first bubble output began at 700 mmHg and continued for 5 days (Table IV). We developed the active and passive forced impregnation protocol proposed by Ottone *et al.* (2015). The specimens were kept in the S10/S3 mixture for 1 day at room temperature after making sure that acetone output (bubble output) ended in both groups. Then, the specimens taken out of the tanks continued to be kept on the filters for excess silicone to be released at room temperature for 7 days. The remaining silicones were taken by using lint-free papers, and the gas curing phase began.

Table I. Acetone input and output concentrations in dehydration stage of undetected sensory organs.

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Acetone	Input	Output	Fixed		
Bath	(Acetone	(Acetone	Time		
	concentration)	concentration)			
Bath	% 99.5	% 84	8 th day		
Bath	% 99.5	% 92	^{6th} day		
Bath	% 99.5	% 98	5 th day		

Table II. Acetone input and output concentrations in the dehydration stage of the sensory organs detected.

0	5 0		
Acetone	Input	Output	Fixed
Bath	(Acetone	(Acetone	Time
	concentration)	concentration)	
Bath	% 99.5	% 87	7t ^h day
Bath	% 99.5	% 96	7t ^h day

Table III. Distribution of pressure level of bubble onset according to days in forced impregnation of sensory organs detected.

Day	Forc ed impregnation	Pressure (mmHg)
1. Day	8 saat active	640
-	16 saat passive	760
2. Day	8 saat active	680
	16 saat passive	760
3. Day	8 saat active	720
	16 saat passive	760
4. Day	8 saat active	760
	16 saat passive	760
5. Day	8 saat active	760
	16 saat passive	760
6. Day	8 saat active	760

Table IV. Distribution of pressure level of bubble onset according to days in forced impregnation of undetected sensory organs.

Day	Forced impregnation	Pressure (mmHg)
1. Day	8 hours active	700
	16 hours passive	760
2. Day	8 hours active	720
	16 hours passive	760
3. Day	8 hours active	740
-	16 hours passive	760
4. Day	8 hours active	760
	16 hours passive	760
5. Day	8 hours active	760
	16 hours passive	760

The specimens taken into the gas curing unit were kept at certain intervals every day in the gas phase of the BIODUR S6. The specimens undergoing formalin fixation were observed to have hard surface and dried silicone on day 6. The specimens that did not undergo formalin fixation were observed to have hard surface and dried silicone on day 4.

In both groups undergoing silicone plastination at room temperature, plastinates were very similar to their natural appearance and maintained their morphological properties. It was qualitatively observed that the natural color was preserved in the group that did not undergo fixation compared to the group undergoing fixation. Moreover, volumetric shrinkages were observed qualitatively in the group undergoing fixation.

In the nose, which is the olfactory organ, the shape of nasus externus and nares kept its normal structures (Fig. 1). Planum nasolabiale was distinct. Septum nasi was very



Fig. 1. Lateral view of nasus externus and nares.



Fig. 2. Septum nasi.

pronounced, and concha nasalis (dorsal, medial, ventral) and meatus nasi (dorsal, medial, ventral) were easily visible (Figs. 2 and 3). The plicae (recta, alaris, basalis) at the tips of conchae maintained their shapes (Fig. 3). The sinuses also kept their own structures.

In the tongue, which is the gustatory organ, all structures were preserved, and apex linguae, corpus linguae, radix linguae, torus linguae, fossa linguae and papillae were clearly visible. Moreover, the holes of caruncula sublingualis and organum orobasale were also distinct (Fig. 4).



Fig. 3. Concha nasalis dorsalis, concha nasalis medius, concha nasalis ventralis and meatus nasi dorsalis, meatus nasi medius, meatus nasi ventralis.

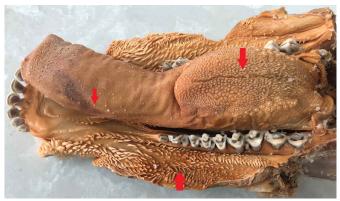


Fig. 4. Linguae and papillae.

RESULTS AND DISCUSSION

The plastinates obtained at the end of the silicone plastination process performed at room temperature were found to maintain the morphological properties they had before plastination in the 2 groups and provided the accurate anatomical details. As pointed out in previous research studies (Latorre *et al.*, 2001; Sagoo & Add, 2013; Ottone *et al.*, 2015; Ekim *et al.*, 2017), plastination provides dry, odorless, and durable

specimens. One drawback of plastination is the reduction of the volume of specimens due to shrinkage. Shrinkage of 10 % is inevitable (von Hagens, 1985). A group of researchers (Pendovski *et al.*, 2004; Sagoo & Adds, 2013) believe that formalin fixation has a negative impact on the color and volume of tissues.

Among the plastinates created in this study, the shrinkage observed in the specimens that did not undergo the fixation phase was found to be much less qualitatively compared to the specimens undergoing fixation. Moreover, the natural colors of the specimens passing through the fixation phase were better protected than those of the specimens on which fixative substance was used. Although fixation is not an important step for silicone plastination, according to the results obtained from on mammals (Ekim et al., 2014a), reptile specimens (Ekim et al., 2017) and snakes (Ekim et al., 2014b), undergoing the fixation procedure have been reported to have better results than specimens that did not undergo the fixation procedure. In our study, however, it was observed that there were no differences other than the difference in the duration of the plastination, considering the fact that the silicon plastination period was completed in 33 days and that of the other group was completed in 31 days.

Acetone baths were performed at -25 °C during the dehydration phase, and no differences were observed in our groups other than the duration. Other researchers (von Hagens, 1985; Ekim *et al.*, 2014b) have also pointed out that it is more appropriate to perform the dehydration phase at -25 °C, as acetone triggers dehydration as well as degreasing at room temperature. It has also been reported that dehydration at low temperatures prevents shrinkage (Henry *et al.*, 1997).

Pendovski *et al.* (2004) and Wendel *et al.* (2008) have reported that the degreasing phase maintains the morphology of other anatomical structures of the tissue and stated that the degreasing phase may be omitted. In our study, the degreasing phase was implemented, and no troubles were observed.

Ekim *et al.* (2017) have reported that the duration of impregnation implemented at room temperature is shorter than the duration of impregnation implemented at low temperatures. Sagoo & Adds (2013) have pointed out that dehydrating specimens at low temperatures and impregnating them at room temperature are more appropriate and yield good results. Our study is in line with these studies (Sagoo & Adds, 2013; Jia-Nan *et al.*, 2013; McCreary *et al.*, 2013; Shanthi *et al.*, 2015; Ekim, 2018).

The silicone-catalyst mixture and the duration of impregnation vary in a number of studies. Raoof et al. (2007) applied the silicone-catalyst mixture at room temperature at the rate of 100:10 (silicone:cross-linker and chain extender) in 5 days, Henry (2007) applied it at room temperature at the rate of 100:8 (silicone:cross-linker) in 5 days, and Ekim applied it at temperature at the rate of 1000:5 in the forced impregnation protocol. Ottone et al. (2015) completed the forced impregnation phase in an average of 5-6 days by using the silicone-catalyst mixture at the 100:1 ratio. In our study, we follow what was proposed by Ottone et al. (2015) about the silicone-catalyst mixture, and was used at the same proportion of 100:1 ratio during the impregnation phase of the plastinates created with the use of fixation, and this phase was completed in 6 days. The silicone-catalyst mixture was used at the 100:1 ratio during the impregnation phase of the plastinates created without the use of fixation, and this phase was completed in 5 days.

Ottone *et al.* (2015) has pointed out that implementing impregnation in a slow and controlled way eliminates the shrinkage of tissues. For this reason, Ottone *et al.* (2015) suggest for the first time that an active and passive forced impregnation should be preferred. Our study also utilized active and passive impregnations, and the active and passive impregnations implemented only in the specimens that were fixed with formaldehyde were found to last longer.

During the gas curing phase, the plastinates that were subjected to impregnation at room temperature dried and hardened faster, as many researchers (Sakamoto *et al.*, 2006; Raoof *et al.*, 2007; McCreary *et al.*, 2013) also concurred. The resulting specimens offer the ability to manipulate them in a safe manner without the need for gloves. Curing durations were similar to the durations that were between the techniques of dehydration at low temperature and impregnation at room temperature. In our study, the plastinates that were subjected to impregnation at room temperature showed the same characteristics as those in other studies and the plastinate characteristics did not differ between our groups.

All plastinates that were created in our study maintained their natural appearances in general.

The differences between the plastinates that were created with the use of formalin fixation and those without the use of formalin fixation were found as follows.

- Silicon plastination was completed in approximately 31 days in the specimens undergoing the fixation process, and in an average of 33 days in the specimens that did not undergo the fixation process.

- The rate of shrinkage was greater in the plastination of the specimens undergoing the fixation process. However, the rate of shrinkage was observed to be in a very small amount among the specimens that did not undergo the fixation process. Except for these differences, all plastinates had natural appearance.

CONCLUSIONS

There was no significant difference between the silicone plastination cases with and without the implementation of the fixation process. This result makes it possible for us to completely remove fixatives, which are carcinogenic and toxic, from our lives. We believe that this technique will protect cadavers without formaldehyde toxicity and will help to obtain the best quality specimens. We have also observed that plastinates can serve a variety of purposes, including morphology and anatomy, as well as different branches of science. They can be used in surgery, clinic, pathology and diagnostic imaging, as well as for educating students and helping them practice.

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BAYGELDI, S. B.; OZKAN, Z. E.; YI'LMAZ, S.; ASLAN KANMAZ, Y. Plastinación de silicona de nasus y lengua en rumiantes mayores con y sin el uso de formaldehido. *Int. J. Morphol.*, *40*(*4*):966-972, 2022.

RESUMEN: La plastinación es una técnica de preparados anatómicos caracterizada por la sustitución de fluidos tisulares por un polímero reactivo. A pesar de ser económicamente más costoso que muchas métodos anatómicos, este técnica es deseable debido a que las muestras creadas son muy similares a la apariencia natural de los objetos previstos y son productos finales duraderos e inofensivos para la salud humana. Nuestro objetivo principal fue dejar completamente de lado el formaldehído y las sustancias químicas cancerígenas similares utilizadas en un método como la plastinación y permitir la producción de plastinados libres de formaldehído para su uso en la formación y los exámenes de anatomía en nuestro país. Con ese fin, comparamos la nariz y la lengua de 10 rumiantes mayores sometiéndolos a plastinación, 5 de ellos con formaldehído y 5 de ellos sin formaldehído, y buscamos eliminar el formaldehído considerando la diferencia entre ellos. La plastinación con silicona es la técnica más utilizada y más conocida entre las técnicas de plastinación porque los especímenes creados con ella se ven estéticamente impresionantes. La plastinación con silicona consta principalmente de 5 fases. En primer lugar, obtuvimos el situs anatómico que queríamos y preparamos los especímenes diseccionando algunos de ellos después de la fijación y otros sin fijación. Luego, de la implementación de una fase de deshidratación en baños de acetona a -25 °C, se implementó una fase de impregnación forzada utilizando una mezcla del químico S10-S3 a presión negativa. En la fase final, la fase de curado y endurecimiento, se completó el proceso de plastinación dando a los especímenes su forma definitiva con el uso de la solución S6. Como resultado, no se observaron diferencias significativas entre la plastinación con silicona con y sin formaldehído.

PALABRAS CLAVE: Anatomía; Formaldehído,; Rumiantes mayores; Plastinación; Silicona.

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