ORIGINAL RESEARCH PLASTINATION

Plastination of Specimens Using the Silicone (S10) Technique Following Embalming with Dodge© Products Results in Improved Color Retention and Tissue Differentiation

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ABSTRACT:
Embalming and plastination are both processes which tend to reduce the amount of natural color within a specimen. This can result in a lack of definition between tissue types making them less effective as teaching resources. The Mazwell Group© manufactures and distributes Dodge© embalming chemicals for the funeral industry which are intended to give a life-like appearance. The Royal Veterinary College (RVC) has begun to use these chemicals as standard for their anatomical embalming. However, there is conflicting literature on whether these types of chemicals are suitable precursors to plastination, a process which the RVC also carries out. Bovine, canine, and equine hearts were plastinated following removal from cadavers embalmed using Dodge© embalming chemicals. The final specimens were compared both visually and with an RGB color sampler, with those plastinated following embalming with a standard formalin/water/glycerol solution. Comparisons of the heart specimens and others showed an increase in color retention and tissue differentiation. The use of Dodge© embalming chemicals has been shown not to be detrimental to the plastination process and results in specimens suitable for anatomical teaching.

KEY WORDS: anatomy; embalming; hearts; plastination; veterinary

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Introduction

Embalming is an ancient process that has evolved considerably through time. Today, it is not only valuable as a part of the funeral industry, but also as a part of medical and veterinary anatomical education. Plastination, by contrast, is a relatively modern process, having been developed in the late 20th century (Henry et al., 2019), and is still evolving. Embalming and plastination are both methods of preservation, vastly different in their processes, but one is often necessary for the other to take place. The Royal Veterinary College (RVC) has a plastination laboratory which produces a wide range of specimens for teaching and widening participation activities. The system in use at the RVC is the cold-temperature silicone standard S10 technique, and requires the specimens to be embalmed in the first instance (Henry *et al.,* 2019).

In the last few years, the RVC has transitioned from using a simple formalin/water/glycerol (FWG) solution to using embalming chemicals originally intended for the funeral industry, the reason being that they improved upon the primary criteria of longevity, color retention and ease of specimen storage. The Mazwell Group©, which produces the Dodge© embalming chemicals (The Mazwell Group 2020a) now used at the RVC, has a wide range which can be mixed to achieve the desired results. One of the chemicals selected from the range, Introfiant (The Mazwell Group 2020b), contains a dark reddish/pink stain called Dynachrome which gives the cadavers a natural appearance in terms of color. When the RVC transitioned to Dodge© embalming chemicals the outcome of how they would perform in conjunction with the plastination process was unknown. This technical report will describe: the chemicals and the process used to embalm canine, equine, and bovine cadavers; the process used to plastinate the isolated hearts taken from the embalmed canine, equine and bovine cadavers; and the results following plastination.

Materials and Methods

Embalming

Three cadavers were used in this study: bovine, canine, and equine. All three were sourced in accordance with the RVC's ethical guidelines. The chemicals used to embalm these cadavers are described in Table 1. The embalming solution is made up in batches of 10 liters.

Table 1: The Dodge© embalming chemicals making up the Royal Veterinary College's 10 Liter solution

Fluid Name	Constituents %	Volume
Rectifiant (Dodge SDS 2020d)	Propylene Glycol 1-10% Trisodium EDTA 1-10%	2 Litres
Metaflow (Dodge SDS 2020c)	Propylene glycol 10-25% Trisodium EDTA 1-10% Methane sulfinylbis 1-10%	3 Litres
Restorative (Dodge SDS 2020e)	Polyoxypropylene glycol 1-10% Propylene glycol 1-10% Polyethylene glycol 1-10% Isopropyl alcohol 1-10% Lanolin, ethoxylated 1-10% Trisodium EDTA	1 Litre
Dis-Spray (Dodge SDS 2020a)	Isopropyl alcohol 50-75% Triethylene glycol 1-10%	1 Litre
Introfiant OTS (Dodge SDS 2020b)	Formaldehyde 25-50% Borax 1-10% Propylene glycol 1-10% Methanol 1-10%	3 Litres

Each cadaver used a different volume of the solution, depending on the species and size (Table 2). The Dodge© embalming machine (The Mazwell Group 2020c) has the ability to vary pressure and flow rate, and select between pulse and continuous flow, all of which are important when embalming cadavers of varying sizes.

The cadavers were cannulated at the carotid artery, and the embalming solution was introduced at a pressure of approximately 965 kPa (140 psi). The flow rate chosen depended on the size of the cadaver (Table 3). A drain was placed in the jugular vein allowing the flushing out of blood. The drain was closed when the majority of the fluid passing through the drain was embalming solution.

Table 2: The volume of embalming solution used in each species

Species	Approximate Weight (Kilograms)	Volume
Bovine	300Kgs	80 Litres
Canine	20-25Kgs	15 Litres
Equine	200Kgs	50 Litres

Table 3: Flow rate of embalming solution used for each species during embalming

Species	Flow rate
Bovine	500-700ml/min
Canine	300-400ml/min
Equine	400-500ml/min

The canine cadaver was embalmed over approximately 3 hours. The equine and bovine cadavers took 6-8 hours. Embalming was considered to be complete when tissues felt fuller to the touch. There was some firming of the tissues, but this effect mostly appeared over the hours/days following embalming. Superficial veins were distended, and there was visible staining of the mucous membranes. The skin was also stained where this was visible, depending on thickness, original color, and hair coverage. Following embalming, the drains and cannulas were removed. The cadavers were washed, wrapped/bagged, and stored in a cold room maintained at approximately 2-5° C for a minimum of 3 weeks before they were used for dissection/prosection.

Following the completion of teaching with the embalmed cadavers, the hearts were all selected as being good enough to retain for plastination. All unwanted tissue was removed from the specimens. They were then sectioned and returned to temporary storage prior to plastination.

Plastination

The cold-temperature silicone standard (S10) technique is the method employed by the RVC. Prior to plastination, the specimens were washed in room-temperature running water for a minimum of 24 hours to remove surface embalming solution and loose tissue or

debris. The stages of plastination used for these heart specimens were as follows:

Dehydration

The specimens were immersed in acetone within stainless steel containers inside a freezer at a temperature of approximately -20°C. The acetone purity was regularly measured at 20˚C using an acetonometer. The acetone was changed to increase the concentration surrounding and within the specimens. Acetone measurements and changes took place on average once a week. Dehydration was considered to be complete when readings were maintained in excess of 98.5% acetone.

Silicone Impregnation

The specimens were transferred to a vacuum chamber and submerged in the liquid silicone (S10+S3) solution. This vacuum chamber resides within a freezer maintained at -20°C, and is connected to a pump. A vacuum was slowly applied to the vacuum chamber with the silicone solution monitored for acetone bubbles. The system at the RVC does not use a manometer. Whenever acetone bubbles subsided, the vacuum was adjusted very slowly using the taps, until bubbles resumed. This was repeated until all taps were closed and all acetone bubbles had ceased rising from the silicone solution. At this point silicone impregnation was considered complete. Impregnation of these specimens took 4-5 months. This is longer than it might have been, however, these specimens were not processed in isolation, being mixed with specimens of different sizes and tissue types. Once impregnation was completed, the vacuum pump was turned off, and the taps opened allowing air to re-enter the vacuum chamber. The specimens remained in the vacuum chamber for a minimum of 24 hours before removing onto drip trays.

The specimens were allowed to rest on drip trays for several days. They were frequently rotated to allow excess silicone to drain from them and were also regularly wiped. When the amount of silicone dripping or exuding from the specimens had diminished considerably, the specimens were ready for curing.

Curing

The specimens were transferred to a curing chamber, where they were exposed to a vaporized curing solution (S6). They were rotated and wiped on a daily basis, to prevent the curing of any remaining pools or drips of silicone on the surface of the specimens. The specimens remained in the curing chamber for several days. Once they were no longer obviously tacky to the touch, the specimens were transferred on to paper towels for several days to check that they did not return to being tacky. If at any point they resumed being tacky, they were returned to the curing chamber for a few more days. Once the specimens had remained dry for several consecutive days, curing was considered complete. Total curing time varied between specimens, with the dog heart curing in approximately 1 week, and the equine and bovine hearts approximately 3 weeks.

Imaging

All photographs of specimens taken for this study were carried out on the same day with the same lighting, background, and camera.

Color Analysis

The colors of the plastinated Dodge© embalmed specimens were compared with those of previous plastinated specimens that had been embalmed with an FWG solution. The colors were sampled using an online RGB color tester (Rapid Tables 2021) which provided the RGB and #HEX values of each color, as well as a basic color name. Photographs of two specimens for comparison were pasted into the color picker. Several regions and tissue types were sampled, with three readings taken in each area to ensure variations in the colors across tissues were accounted for. Wherever possible, areas sampled were those in direct light rather than those in shadow.

Results

The final plastinated heart specimens can be seen in Figures 1-6. Examples of hearts plastinated following the RVC's previous FWG embalming solution can be seen alongside those embalmed with the Dodge© chemicals in Figures 7 and 8. The colors of the bovine and canine specimens shown in Figures 7 and 8 were sampled using the Image Color Picker (Rapid Table 2021). There were no plastinated FWG embalmed equine specimens available for comparison.

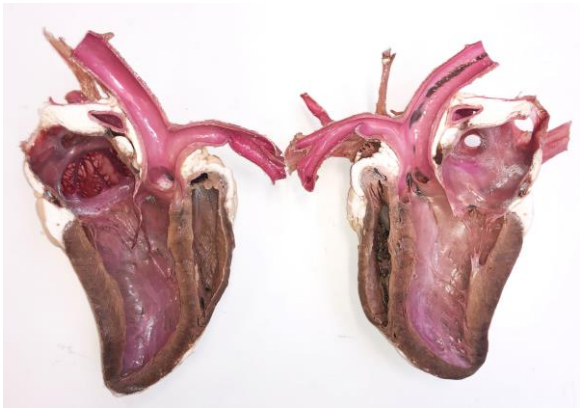

Figure 1. Plastinated bovine heart, sectioned surfaces. Embalmed with Dodge© chemicals

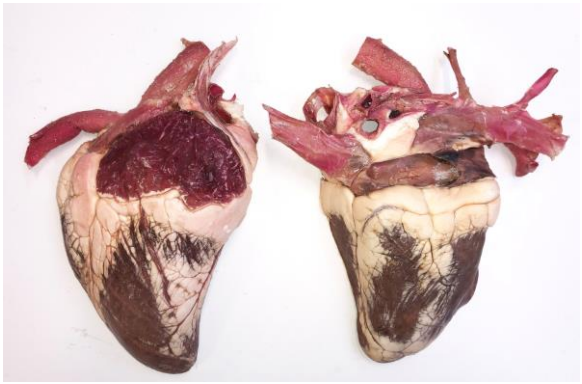

Figure 2. Plastinated bovine heart, outer surface. Embalmed with Dodge© chemicals

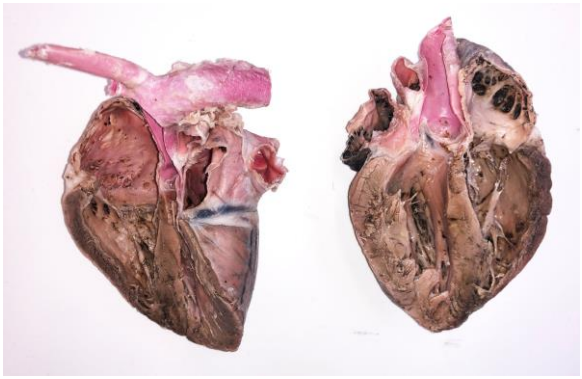

Figure 3. Plastinated canine heart, sectioned surfaces. Embalmed with Dodge© chemicals

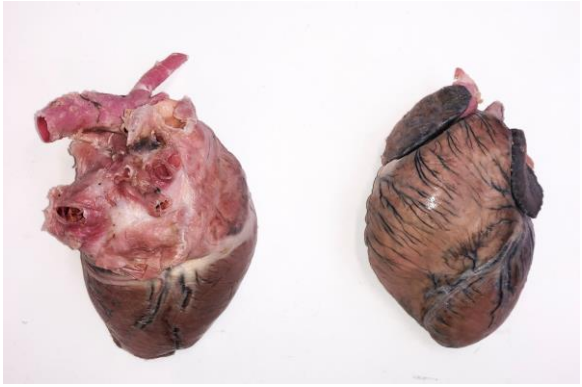

Figure 4. Plastinated canine heart, outer surfaces. Embalmed with Dodge© chemicals

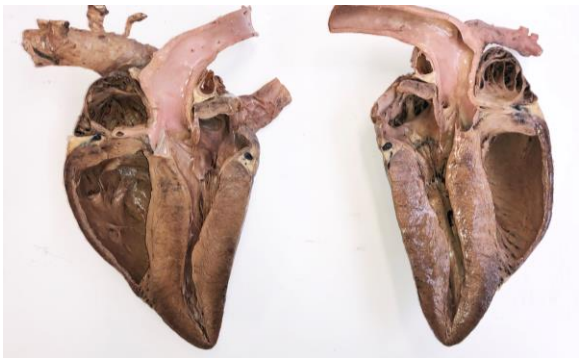

Figure 5. Plastinated equine heart, sectioned surface. Embalmed with Dodge© chemicals

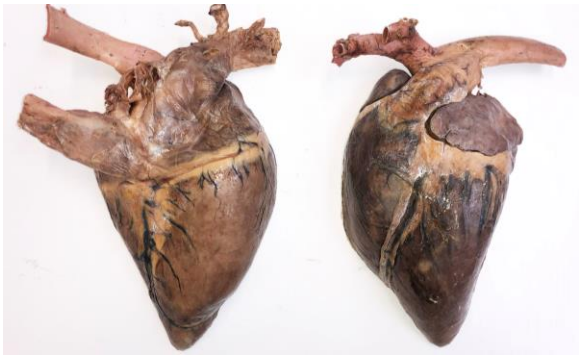

Figure 6. Plastinated equine heart, outer surface. Embalmed with Dodge© chemicals

Figure 7. Plastinated bovine hearts, outer surfaces. The specimen on the left was embalmed with a traditional formalin/glycerol/water solution. The specimen on the right was embalmed with Dodge© chemicals

Figure 8. Plastinated canine hearts, outer surfaces. The specimen on the left was embalmed with a traditional formalin/glycerol/water solution. The specimen on the right was embalmed with Dodge© chemicals

Table 4 shows the color sampling of four tissue types across both plastinated bovine hearts (Figure 7). Three samples were taken in each area, and the median RGB and color name for each region was calculated. The FWG embalmed bovine heart had colors in the grey and silver categories, whereas the Dodge© embalmed bovine heart had colors in the maroon, purple, grey, and white categories. Table 5 shows the color sampling of three tissue types across both canine plastinated hearts (Fig. 8). Again, three samples were taken in each area, and the median RGB and color name for each was calculated. The FWG embalmed canine heart was found to have colors which were categorized as variations of grey across all tissue types. The Dodge© embalmed canine heart was found to have a combination of grey and purple colors across the three tissue types sampled.

Analysis of the colors of these specimens showed that the FWG embalmed specimens have colors within the grey and silver spectrum, whilst the Dodge© embalmed specimens seem to have a wider range of colors across tissue types.

Table 4: RGB Analysis comparing of the Dodge© embalmed plastinated bovine heart with a formalin/water/glycerol embalmed plastinated bovine heart

Tissue/Structure	Dodge© Embalmed					Formalin/Water/Glycerol Embalmed				
	RGB			#Hex	Colour Name	RGB			#Hex	Colour Name
	Red	Green	Blue			Red	Green	Blue		
Myocardium	80	56	50	#503832	maroon	114	99	88	#726358	grey
	74	49	44	#4A312C	maroon	75	60	56	#4B3C38	maroon
	89	65	53	#594135	olive	116	99	90	#74635A	grey
Median Color	*81*	*57*	*49*	*#513931*	*maroon*	*102*	*86*	*78*	*#66564E*	*grey*
Auricles	86	39	47	#56272F	maroon	199	166	199	#C7A677	silver
	121	57	66	#793942	purple	151	117	89	#977559	grey
	115	62	80	#733E50	purple	157	110	68	#9D6E44	grey
Median Color	*107*	*53*	*64*	*#6B3540*	*purple*	*169*	*131*	*119*	*#A98377*	*grey*
Aorta/brachiocephalic artery/pulmonary trunk	177	98	106	#B1626A	grey	220	199	182	#DCC7B6	silver
	170	90	93	#AA5A5D	grey	236	219	186	#ECDBBA	silver
	169	65	81	#AA5A5D	grey	228	204	182	#E4CCB6	silver
Median Color	*172*	*84*	*93*	*#AC545D*	*grey*	*228*	*207*	*183*	*#E4CFB7*	*silver*
Coronary fat	248	235	233	#F8EBE9	white	253	250	239	#FDFAEF	white
	243	206	194	#F3CEC2	silver	205	177	141	#CDB18D	silver
	243	224	222	#F3E0DE	white	242	234	215	#F2EAD7	white
Median Color	*245*	*222*	*216*	*#F5DED8*	*white*	*233*	*220*	*198*	*#E9DCC6*	*silver*

Table 5: RGB Analysis comparing of the Dodge© embalmed plastinated canine heart with a formalin/water/glycerol embalmed plastinated canine heart

Tissue/ Structure	Dodge© Embalmed					Formalin/Water/Glycerol Embalmed				
	RGB			#Hex	Color Name	RGB			#Hex	Color Name
	Red	Green	Blue			Red	Green	Blue		
Myocardium	146	107	90	#926B5A	grey	187	164	155	#BBA49B	silver
	114	73	65	#724941	grey	128	106	96	#806A60	grey
	135	93	86	#875D56	grey	107	80	69	#6B5045	grey
Median Color	*132*	*91*	*80*	*#845B50*	*grey*	*141*	*117*	*107*	*#8D756B*	*grey*
Auricles	72	62	73	#483E49	purple	195	178	171	#C3B2AB	silver
	67	58	68	#433A44	purple	135	111	106	#876F6A	grey
	74	64	72	#433A44	grey	140	120	114	#8C7872	grey
Median Color	*71*	*61*	*71*	*#473D47*	*purple*	*157*	*136*	*130*	*#9D8882*	*grey*
Aorta/ Brachiocephalic artery/ Pulmonary trunk	165	107	124	#A56B7C	grey	167	138	124	#945E71	grey
	189	142	160	#BD8EA0	silver	163	139	135	#A38B87	grey
	148	94	113	#945E71	grey	169	150	146	#A38B87	grey
Median Color	*167*	*114*	*91*	*#A7725B*	*grey*	*166*	*142*	*135*	*#A68E87*	*grey*

Even without the RGB color analysis it can be easily seen in Figures 1-8 that the heart specimens embalmed with the Dodge© chemicals have, to varying degrees, retained the artificial color introduced at the embalming stage. The bovine heart appears to have retained the most color, and the equine heart the least. The reasons for this variation in color retention across the three examples is unknown. Visual comparisons were also made with plastinated specimens from different body regions and tissue types (Figs. 9-11), embalmed with the Dodge© and FWG solutions. These also demonstrated a similar trend, with the Dodge© embalmed specimens exhibiting a wider range of colors.

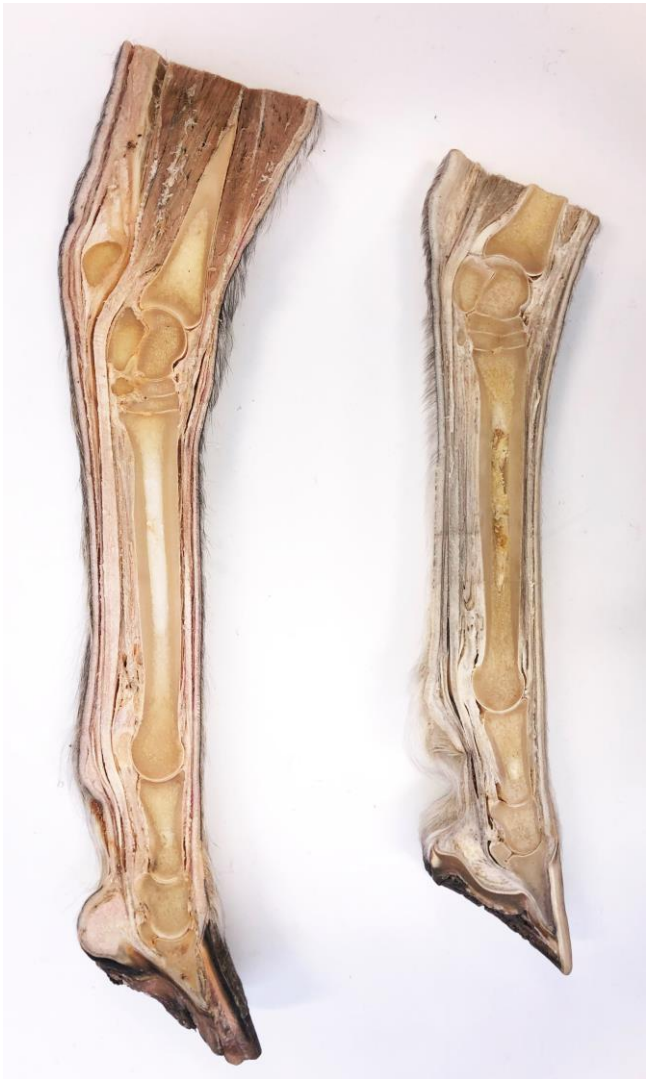

Figure 9. Plastinated equine distal hindlimb. The specimen on the left was embalmed with Dodge© chemicals. The specimen on the right was embalmed with a traditional formalin/glycerol/water solution

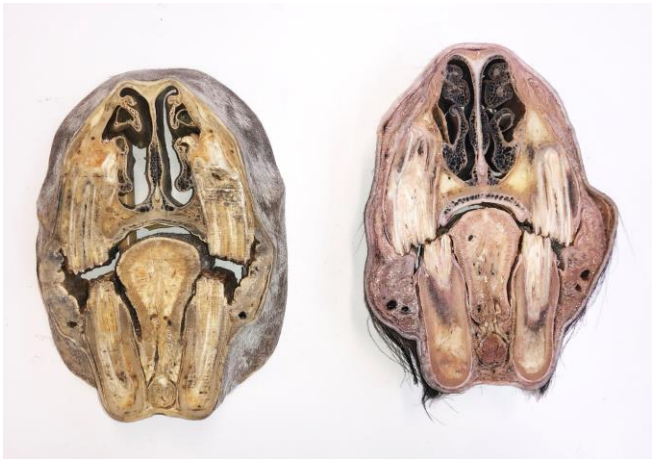

Figure 10. Plastinated transverse section of the equine head at the level of the nasal cavity. The specimen on the left was embalmed with a traditional formalin/glycerol/water solution. The specimen on the right was embalmed with Dodge© chemicals

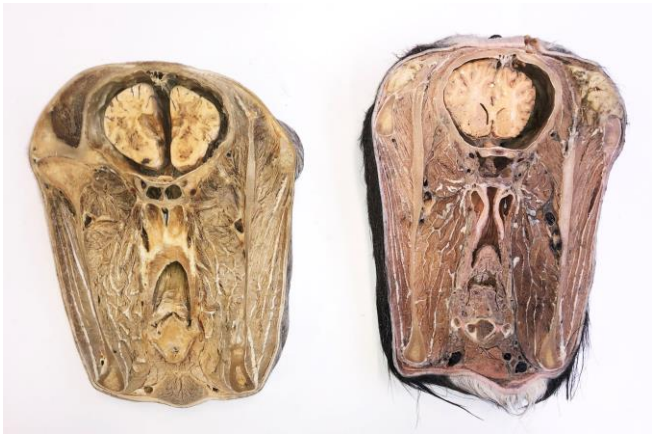

Figure 11. Plastinated transverse section of the equine head at the level of the cranium. The specimen on the left was embalmed with a traditional formalin/glycerol/water solution. The specimen on the right was embalmed with Dodge© chemicals

When all these specimens were photographed, they were at least 18 months post-curing, some considerably more so. There appeared to be no deterioration in the Dodge© specimens, including any obvious changes in color or shape. There was a small amount of white dust accumulated on the surface of two of the specimens, visible in Figures 1 and 6, but this was easily wiped away with a dry piece of tissue.

An unexpected result of the process described was that, during dehydration of the Dodge© embalmed specimens, it was observed that the acetone leached some of the red stain from the specimens. This did not appear to

have any detrimental effect on the process itself, or the final result. It was, however, also observed that other specimens embalmed with just formalin/water/glycerol that went through the dehydration process at the sametime, seemed to take on a little of the red stain. Again, this did not appear detrimental to these specimens and may have even improved upon their appearance.

Discussion

Traditional embalming solutions used for specimens intended for plastination usually include formalin at 5-15% (Henry et al., 2019), however, higher concentrations of 10-20% should be used for brains (Henry et al., 1997). Based on the constituent concentrations shown in Table 1, the RVC's embalming solution formalin concentration ranges between 7.5% - 15%. Whichever concentration of formalin chosen, there are differing opinions on whether the addition of further chemicals to the embalming solution is beneficial or disruptive to the plastination process. Some believe embalming solutions containing alcohols, glycerin, glycols and/or phenol should not be used on specimens destined for plastination (DeJong and Henry, 2007). Others, however, have used solutions containing one or more of these chemicals and are satisfied with the results (Cook and Dawson, 1996; Pretorius, 1996; Norman and Nicoll, 2017).

Some authors have gone down the route of having two separate embalming solutions, one for dissection specimens, and one specifically for specimens intended for plastination (Cook and Dawson, 1996). This would not be a suitable option for the RVC, as the majority of the specimens plastinated are those which are initially either used as student dissection specimens during undergraduate teaching, or as prosections produced by staff for demonstration. Therefore, the embalming chemicals selected by the RVC are primarily chosen to provide teaching specimens which are not only long lasting and easy to store, but also provide good color and tissue differentiation during dissection. Good quality student dissection or prosection specimens may then be retained once finished with, for plastination. Only a very small number of RVC specimens are ever embalmed with plastination being the primary goal.

Most embalming solutions are traditionally transparent, which, when used in conjunction with the standard embalming process of draining blood from the cadaver, can produce a very pale grey/beige-looking specimen. This is not ideal, as good tissue differentiation is key in aiding student identification and understanding of anatomical structures. In the past, various embalming solutions were developed to aid the preservation of color e.g., Kaiserling's, Klotz, Jores', and McCormick's solutions, and other coloring chemicals, such as eosin and merthiolate (Iliff et al., 2019). However, these seemed to yield mixed results. Plastination can further bleach the color from specimens during the dehydration stage (McCreary et al., 2013) leaving specimens pale, with features harder to distinguish. Today, in order to achieve a more natural and useful appearance, many plastinated specimens are painted or stained (Raoof et al., 2013; Mccreary et al., 2013; Yu et al., 2014; Kang et al., 2015). A stain could be added to the acetone bath, or a pigmented silicone could be used during the impregnation stage of plastination. These options are unfortunately not selective, so all the tissues will be the same color (Iliff et al., 2019), which would not be an improvement. The ideal would be to have an embalming solution which produces good tissue color differentiation upon dissection, which is also suitable for plastination.

This study has looked at the production of plastinated specimens using a colored embalming solution, however the number of specimens used was limited and further investigations should be made into its wider suitability.

Conclusion

The processes described in this report have successfully produced useful plastinated teaching specimens, and also improved upon the appearance of the final product without having to add any additional stages, such as painting or staining. The greater range of colors found in the Dodge© embalmed specimens, as opposed to FWG embalmed specimens, may aid the observer in differentiating between tissue types.

The switch from using a clear FWG embalming solution to one which contains an artificial color replacement at the RVC does not appear to have been detrimental to the plastination process. The specimens will continue to be observed for signs of deterioration in the longer term, but at this time the RVC continues to use Dodge© chemicals for embalming specimens prior to plastination.

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