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LETTER FROM THE PRESIDENT

Dear Friends,

In this new issue of the Journal of Plastination, I want to thank the authors of the papers, who have chosen our journal to publish their results. Most of them are new members of the ISP. Thank you for joining us; I am sure your enthusiasm in plastination will be reflected in new ideas and results as you have already proved in these papers. These articles allow us to learn about new applications of plastination techniques, and open up new work options in our laboratories. This issue also includes the abstracts of the communications presented at the XII Interim Meeting on Plastination, Durban 2017, South Africa. I want to especially thank Phil, our Editor, for the effort he has made to co-ordinate and publish this new issue of the journal.

As most of you know, the XIX International Conference on Plastination (http://www.icp2018dalian.org ) will be held in Dalian, China, next July. The host is Prof. Hong-Jin Sui and his team from Dalian Medical University. The dates are July 18-23th, 2018, with a pre-conference workshop on plastination. As president of the ISP I would like you all to become actively involved in this conference, sending communications and attending it personally. It will be a great opportunity to share new experiences about innovation and to establish future collaborations for the advancement of plastination. I hope I can meet all of you in Dalian.

Greetings,

Rafael Latorre
President
LETTER FROM THE EDITOR

Plastination: Global Impact on Science, Education, and Culture

Dear Colleagues,

Proof, if proof were needed, of the wide range of applications, durability, and global impact of plastination is amply demonstrated in this issue of the Journal of Plastination. The papers published here cover topics as diverse as marine biology, archaeology, parasitology, and conservation of plastinates. In “The Challenges of Plastinating a Blue Whale (Balaenoptera musculus) Heart” Miller et al. describe a notable achievement, which is of massive cultural, educational, and scientific significance. The whale in Miller et al.’s paper was 23 metres in length, while at the other extreme, González et al. describe the application of plastination, on a very much smaller scale, to the preservation of the larvae of Oestrus ovis (the sheep bot fly), a mere 1.5 cm in length. There are very few studies in the literature on the application of plastination to parasitology: this paper is a welcome addition to the field.

Plastination has also been used to conserve archaeological artefacts, and in this issue we publish a paper by Buendía et al. on the application of plastination to the field of marine archaeology. In their paper “Plastination Applied to the Conservation of Cultural Heritage” the authors describe the preservation of very significant ivory artefacts recovered from a Phoenician ship that sank off the coast of Spain between 7th – 6th centuries BC. And, as Miller et al. write: “The longevity of plastinates is advantageous for preservation biological tissue, but especially rare or unique specimens of inherent scientific interest” (emphasis added), which describes perfectly the various, very different, specimens that form the focus of these three papers.

However, while longevity is one of the hallmarks of plastination, repeated use in teaching inevitably leads to damage. It is our experience at St George’s, University of London that arteries are particularly brittle, and repairs are not always satisfactory. Plastinated specimens, by their very nature, are ideal for students to handle and inspect for themselves: the dilemma we face as educators is whether to allow students to handle them and risk damage, or restrict access so that they last longer, but deny students the important “hands-on” experience. This issue is discussed by Johnson and Baker in their paper “Rehabilitation of Plastinated Anatomical Prosections Using Silicone Adhesive and Pre-Cured S10/S3-Impregnated Fascia and Muscle”, in which they describe an ingeniously use of silicone-impregnated, but as yet uncured, tissue, to repair damaged and broken specimens.

In the controversy that surrounded the first ‘Body Worlds’ exhibition in the UK, in 2002, Gunther von Hagens was demonised in the popular press, and portrayed as being a modern-day Dr Frankenstein (Harris & Connolly, 2002). Interestingly, the two countries where von Hagens encountered the strongest opposition to his Body Worlds exhibitions...
were the UK and Germany (von Hagens, 2008.) In the UK there were, at that time, heightened sensibilities following the revelations of unlawful retention of thousands of organs from babies and children at post mortem. This scandal ultimately led to a change in the law, and the introduction of the Human Tissue Act (Human Tissue Authority, 2018), which has helped to rebuild public confidence to the point where exhibitions of human and animal plastinates have proved hugely popular with the public, and body donations to anatomy have recovered from their post-scandal slump. In Germany, opposition to the display of human bodies appears to have been as a result of uncomfortable associations with the Third Reich. Anatomists and historians are now beginning to address the Nazi era; the careers of prominent anatomists of that time, such as Eduard Pernkopf (Hildebrandt, 2006) and Hermann Stieve (e.g. Winkelman & Schagen, 2009; Hildebrandt, 2013), to whom bodies were delivered fresh from the executioner, are now being analysed and discussed.

In these thankfully more enlightened times, who can now doubt the positive contribution that plastination has made to education, science, and culture?

With best wishes,

Philip J Adds
Editor-in-Chief

References


S10 Plastination Technique for Preservation of Parasites: the case of *Oestrus ovis* larvae

**ABSTRACT:**
Plastination is a method of preserving biological tissue in a dry, odorless state, avoiding the use of traditional chemicals, some of them carcinogenic, such as formaldehyde. In recent years, plastination has been introduced in many anatomy departments of human and veterinary medicine, where plastinated specimens are used as a teaching tool for gross anatomy. There are very few references in the literature describing the plastination of parasitic specimens. Formalin-fixed *Oestrus ovis* larvae, stages L2 and L3, were dehydrated in acetone, and plastinated with the standard Biodur® S10 protocol. Plastinated larvae suffered a collapse of their structures, with a macroscopic deformation of their appearance. In a second test, making an incision in the ventral part of the cuticle allowed us to obtain, for the first time, plastinated larvae of *Oestrus ovis*, preserving the morphological characteristics that these specimens had before the plastination process.

**KEY WORDS:** Cuticle, *Oestrus ovis*, Parasitology, Plastination, S10 technique

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**Introduction**
Nowadays, conservation of parasitic specimens for teaching or research is necessary. However, the most commonly used preservatives (formaldehyde and alcohol) have disadvantages, such as toxicity, carcinogenicity, odor, constant preservative maintenance, storage space, etc.

The technique of plastination (Von Hagens et al., 1987) is generally presented as the most recent and important option for conservation of biological material, which is demonstrated by its rapid expansion in recent years. Plastination consists of processes whereby tissue fluids and fat are slowly replaced by a curable polymer under vacuum conditions. This technique allows us to obtain clean, dry, resistant preparations of unlimited duration, which can be examined without gloves or any other type of protective equipment, and do not require any special treatment or storage conditions. It also avoids the daily exposure of students and teachers to harmful products because they are free of toxic substances, such as formaldehyde, phenol, alcohols, etc. (McLaughlin, 1994; Swenberg et al., 2013).

Plastinated organs enhance the quality of the teaching-learning process at different educational levels in secondary (biology, zoology), and university (anatomy, pathological anatomy, etc.) (Latorre et al., 2007). However, there are very few studies that refer to the conservation of parasites using plastination techniques for teaching or research purposes (Asadi & Mahmodzadeh, 2004; Kocevski et al., 2010; Essa et al., 2014). Preliminary results from studies on plastination techniques applied to parasitology show that each parasite from each of the different taxa needs a specific plastination protocol, in order to preserve their particular morphological and structural characteristics (Grondin et al., 1994).

The objectives of this study were: 1) to evaluate the morphology and morphometry of *Oestrus ovis* larvae, stages L2 and L3, preserved with the standard S10 plastination protocol; and 2) to establish the necessary changes in the protocol to improve the final results of the plastinated specimens.

**Materials and Methods:**
Twenty *Oestrus ovis* larvae from the collection of the Animal Health Department, University of Murcia, Spain, were used in this study, divided in two assays (Fig. 1). For the first trial, ten formalin-fixed larvae (two immature or L2, and eight mature or L3) were used. All larvae were...
individually evaluated to identify and discard specimens with anomalous features, or structural alterations. Each specimen was individually measured to obtain baseline morphological parameters, in order to be able to evaluate changes during each phase of the process.

Figure 1a and 1b

For this evaluation, a photomicroscopy system was used (Leica EZ Camera 2.4.1) and a specific morphometry software (LAS EZ 2.1.0). The parameters measured were: length (cranio-caudal), maximum body width, minimum width (distance between antennal lobes), and weight. These parameters were recorded after fixation, dehydration, impregnation, and curing.

The standard Biodur® S10 silicone technique was used (DeJong & Henry, 2007). Dehydration was carried out by freeze substitution with acetone (-25° C); forced impregnation was carried out at -25° C under vacuum using silicone S10 and catalyst S3 (100:1) as the reaction mixture. Curing was performed at room temperature using vaporized S6 cross-linker.

Histological cross sections from plastinated larvae were obtained to determine potential structural alterations associated with the process. One mature specimen, and other immature larvae from each assay were used for the histological study. As a control group, different to the two assays, a fixed mature larva and other fixed immature larvae were used. A de-plastination protocol (Grondin et al., 1994) was necessary in order to obtain histological sections from the plastinated larvae.

A second experiment was necessary to improve on the results obtained from the first test. In this second assay, ten additional larvae (five immature L2, and five mature L3) were used. The conventional protocol was modified, making a longitudinal incision of approximately one centimeter on the belly of the parasite, to prevent the cuticle becoming a barrier during acetone and impregnation mixture exchange. As in the previous experiment, the morphometric parameters were monitored to analyze potential structural variations.

Parameters evaluated in both experiments were analyzed for statistical differences using a non-parametric Wilcoxon test. Significance was set to p <0.05.

Results

First assay

Plastination protocol: Dehydration by cold substitution of acetone took approximately 5 days, with a total of three baths (Table 1). From the 5th day, the purity of the acetone did not fall below 99.8%. Even so, the specimens were kept for 6 more days in the last bath, to compensate for the possible retardation of dehydration by the cuticle. Impregnation in the S10 + S3 solution did not present any problems, and it took 5 days to reach a final pressure of 5 mmHg, and to achieve the cessation of bubbling of acetone vapor. At the end of impregnation, the samples were removed from the impregnation chamber to room temperature for 24 hours before initiating the polymerization process. The Oestrus ovis specimens were left in the curing chamber while removing excess silicone from the surface. During the curing phase, the organisms were removed from the chamber and stored at -20° C, when excessive surface silicone was not able to be controlled, such as at night. In total, the specimens remained in the polymerization chamber for 24 days (Table 1).

<table>
<thead>
<tr>
<th>Assay</th>
<th>Dehydration (days)</th>
<th>Impregnation (days)</th>
<th>Cured (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>first assay</td>
<td>11</td>
<td>5</td>
<td>24</td>
</tr>
<tr>
<td>second assay</td>
<td>7</td>
<td>15</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 1. Differences in the duration of different stages in both assays.

Morphological results: The results obtained in this first test were not satisfactory. Only one specimen retained the appearance of its initial state. During dehydration and impregnation, no apparent morphological problems occurred, but during the curing process, the specimens suffered a severe collapse, with alteration of the normal anatomy (Fig. 2a). Two of these plastinated parasites had excessive macroscopic disruption of the cuticular structures, especially in the rows of spines that border each segment of the larva (Fig. 2b). In addition, one of the specimens showed a deposit of multiple small granules of reddish coloration, approximately half a millimeter in diameter, corresponding to silicone waste (Fig. 2c). After curing, it was possible to observe the existence of damaged and/or broken structures, such as the buccal hooks of these parasites (Fig. 2d). Some defects began
to appear in some individuals during the early stages of processing, especially at the end of dehydration, where the fragility of the organisms was evident.

Figures 2a, 2b, 2c and 2d

The histological study, comparing non-plastinated with plastinated larvae, was designed to determine the possible causes of the morphological alterations resulting from the plastination process (Fig. 3). Histological evaluation of the fixed larvae permitted appreciation of the great thickness of its cuticle (340 μm). Histological images of larvae (L1 and L2) before and after plastination did not show any other alterations that could explain the reason for the collapse that the structures suffered during processing.

Figures 3a, 3b, 3c, 3d, 3e and 3f

Morphometric results: Table 2 shows the variations of the morphometric parameters of the larvae between each phase, and in the total plastination process, expressed as a percentage of the initial value for all variables studied (length, width and weight). As shown in the table, the final result was a decrease in length of both types of larvae, together with an increase in the maximum width of the individuals. However, there was an increase in the minimum width of the L2, unlike the L3, which presented a decrease in this parameter. These variations in width corresponded to morphological deformations on the surface of these parasites, as a consequence of their collapse after curing. The variable that showed the greatest decrease, with significant differences, was the weight, which could be attributed to a failure in dehydration or impregnation. Graph 1 (L2) and Graph 2 (L3) show the variation of the four variables measured during processing of the samples. There is evidence of unequal behavior between the L2 and L3 larvae in their maximum and minimum widths. After several ascending and descending oscillations, both L2 and L3 larvae increased their maximum width at the end of the process. The same happened for L2 with minimum width, whereas L3 larvae decreased for this parameter. With respect to length and weight, a progressive decrease of these parameters occurred during all stages of the plastination process.

None of the p-values analyzed by the statistical study was significant for Oestrus ovis immature larvae (length, maximum width, minimum width and weight); weight changes in mature larvae were significant (p-value < 0.05).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Larva</th>
<th>Initial</th>
<th>Dehydration</th>
<th>Impregnation</th>
<th>Curing</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>L2</td>
<td>+2.38%</td>
<td>0%</td>
<td>-4.65%</td>
<td>-2.38%</td>
<td>0.577</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>-3.02%</td>
<td>-0.28%</td>
<td>-4.26%</td>
<td>-7.42%</td>
<td>0.106</td>
<td></td>
</tr>
<tr>
<td>Maximum Width</td>
<td>L2</td>
<td>+3.45%</td>
<td>+2.44%</td>
<td>+10.71%</td>
<td>+6.90%</td>
<td>0.346</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>-4.65%</td>
<td>+2.44%</td>
<td>+6.51%</td>
<td>+4.03%</td>
<td>0.247</td>
<td></td>
</tr>
<tr>
<td>Minimum Width</td>
<td>L2</td>
<td>-18.67%</td>
<td>-1.36%</td>
<td>+31.17%</td>
<td>+8.13%</td>
<td>0.346</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>+5.54%</td>
<td>-8.82%</td>
<td>-13.23%</td>
<td>-16.5%</td>
<td>0.078</td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>L2</td>
<td>+1.67%</td>
<td>+3.28%</td>
<td>-38.89%</td>
<td>-68.01%</td>
<td>0.095</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>-7.62%</td>
<td>-10.32%</td>
<td>-38.85%</td>
<td>-49.33%</td>
<td>0.014*</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Mean variation (L2 and L3) of morphometric parameters for each phase and the total plastination process in Oestrus ovis during the 1st test (results expressed in % on the initial value) and statistical significance (*): Significant values (p < 0.05).
Graph 1. Variation of the 4 parameters measured in the first trial for immature larvae (L2). (A; length, B; maximum width, C; minimum width and D; weight), during the different stages of the process (1; before process, 2; post-dehydration, 3; post-impregnation and 4; post-curing). Y axis represents millimeters or grams for each variable. Box plot shows median, interquartile range (subdivided box) and standard deviation (outside lines) of variables.

Graph 2. Variation of 4 measures in the first trial for mature larvae (L3). (A; length, B; maximum width, C; minimum width and D; weight), during the different stages of process (1; before process, 2; post-dehydration, 3; post-impregnation and 4; post-curing). Y axis represents millimeters or grams for each variable. Box plot shows median, interquartile range (subdivided box) and standard deviation (outside lines) of variables.

Second assay

Plastination protocol: During this test, 95% acetone was employed in the first bath (-20°C) in order to avoid shrinkage of the larvae. Table 1 shows comparative times for the different stages for the first and second tests. Impregnation was prolonged three times longer than in the first assay to ensure complete exchange of acetone by the reaction mixture. The times employed in each step were adapted to the needs of each assay, using objective measures for them (purity of acetone in dehydration, bubbling in impregnation, and excess of silicone in curing).

Morphological results: On the second day of curing, one of the specimens collapsed, with an excessive and rapid exit of silicone from the surface. This situation gave the appearance of an adherent silicone layer that was difficult to eliminate from the ventral surface. At the end of processing, good morphological results were obtained in 9 of the 10 plastinated larvae (Fig. 4). Slight macroscopic variations can be observed in the plastinated individuals, but much less pronounced than those detected in the specimens of the previous test.

Figures 4a and 4b

Histological images from the first trial (Fig. 3c, d) showed the thick cuticle of the larvae and the presence of a high number of enucleated cells in both plastinated and unplastinated parasites, in contrast with the second trial (Fig. 3e, f). This characteristic seems to be a consequence of the thick cuticle already mentioned above. A potential delayed entry of the formaldehyde during fixation could cause a process of cellular lysis that had an impact during all processes. To avoid this defect, larvae should be fixed with an incision in their cuticle.

Morphometric results: Values of the variables measured (expressed as a percentage) are shown in Table 3. An overall decrease in all the parameters, except for the length in L3, were observed for both types of larvae in all phases of the plastination process. Weight decreased after the dehydration of the larvae, and increased after forced impregnation. The difference between results obtained for L2 and L3 before and after being plastinated were not significant, which shows that these plastinated specimens retained their morphology in an acceptable manner, in relation to their initial state (Graphs 3 and 4).
Table 3. Mean variation for each phase and the total plastination process in Oestrus ovis during the 2nd test (results expressed in % on the initial value) and statistical significance (*): significant values (p < 0.05).

<table>
<thead>
<tr>
<th>LARVA</th>
<th>INITIAL (FIXATION - REHYDRATION)</th>
<th>DEHYDRATION</th>
<th>IMPREGNATION</th>
<th>CURING</th>
<th>TOTAL (Initiation - End)</th>
<th>P-VALOR (Initiation - End)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LENGTH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L2</td>
<td>+1.15%</td>
<td>-3.41%</td>
<td>-4.71%</td>
<td>-6.90%</td>
<td>0.174</td>
<td></td>
</tr>
<tr>
<td>L3</td>
<td>+0.95%</td>
<td>+1.42%</td>
<td>-0.93%</td>
<td>+1.43%</td>
<td>0.789</td>
<td></td>
</tr>
<tr>
<td>MAXIMUM WIDTH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L2</td>
<td>+3.68%</td>
<td>-8.47%</td>
<td>0%</td>
<td>-5.10%</td>
<td>0.181</td>
<td></td>
</tr>
<tr>
<td>L3</td>
<td>-1.61%</td>
<td>-0.70%</td>
<td>+1.18%</td>
<td>-1.15%</td>
<td>0.187</td>
<td></td>
</tr>
<tr>
<td>MINIMUM WIDTH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L2</td>
<td>+2.01%</td>
<td>-1.38%</td>
<td>-3.90%</td>
<td>-3.32%</td>
<td>0.0625</td>
<td></td>
</tr>
<tr>
<td>L3</td>
<td>+0.31%</td>
<td>-2.55%</td>
<td>-1.04%</td>
<td>-3.56%</td>
<td>0.187</td>
<td></td>
</tr>
<tr>
<td>WEIGHT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L2</td>
<td>-21.25%</td>
<td>+15.87%</td>
<td>-30.14%</td>
<td>-36.25%</td>
<td>0.058</td>
<td></td>
</tr>
<tr>
<td>L3</td>
<td>-22.84%</td>
<td>+24.80%</td>
<td>-16.99%</td>
<td>-20.06%</td>
<td>0.062</td>
<td></td>
</tr>
</tbody>
</table>

Graph 3. Variation of 4 measurements in the second trial for immature larvae. (1 large, 2 max length, min length and 4. weight), during the different stages of the process (A; before process, B; post-dehydration, C; post-impregnation and D; post-curing). Y axis represents millimeters or grams for each variable. Box plot shows median, interquartile range (subdivided box) and standard deviation (outside lines) of variables.

Graph 4. Variation of 4 measurements in the second trial for mature larvae (1 large, 2 max length, min length and 4. weight), during the different stages of the process (A; before process, B; post-dehydration, C; post-impregnation and D; post-curing). Y axis represents millimeters or grams for each variable. Box plot shows median, interquartile range (subdivided box) and standard deviation (outside lines) of variables.

Discussion

Plastination is a technique of conservation that produces samples of excellent quality and ease of handling, in contrast to the original biological specimens. In the present study, we have analyzed the possible use of plastination in parasitology, a discipline that has hitherto been largely unexplored.

For this objective, we started by employing the standard S10 protocol, adapting the technique by adjusting the times necessary for each phase. It has been possible to obtain correctly plastinated specimens, with no significant morphometric differences from their previous, formalin-fixed state, which are, therefore, very suitable for use in teaching or research. In addition, throughout the process, the times required to complete each phase have been reduced, compared with data published in previous trials, which would mean reductions in production time, and savings in cost of production (Kocevski et al., 2010).

Direct application of the standard S10 protocol did not enable us to produce an adequate final product. In Oestrus ovis larvae, the thickness of the cuticle may have prevented the circulation of fluids during the fixation, dehydration, and impregnation stages. The different level of development of cuticle in immature and mature larvae could be an element which may influence about the collapse of structures, so it seems appropriate to study each larval stage separately. This potential barrier during fixation could be the reason for enucleated cells, as a result of late penetration of formaldehyde, leading to cellular apoptosis. This fact could also be the cause of inadequate penetration of acetone and silicone during the dehydration and impregnation stages, causing a posterior collapse of the whole parasite during curing, favored by the internal spaces (coelom) that are present in the anatomy of these organisms. For this reason, incision of the cuticle is essential for correct plastination of these parasites. In such cases as these, it will be necessary to develop variations in the standard techniques in order to obtain satisfactory results, as has occurred in other studies, to solve the problem of a cuticle that interferes with the exchange of acetone and polymers, in fetuses or reptiles, among others (Ekim et al., 2017; Tiwari et al., 2012). The decreased time of the dehydration process in the second assay was related to the ventral incision of the larvae, because of the ensuing greater and faster contact between acetone and parasite. The increase in the available surface allows greater contact with silicone, increasing the period of impregnation. Curing time was
reduced, to avoid the rigidity of specimens caused by exposure to crosslinker. Morphometric variations found during the second assay were less pronounced than in the first trial, indicating that this was a better protocol for Oestrus ovis larvae.

In conclusion, the morphology and morphometry of Oestrus ovis larvae, stages L2 and L3, can be preserved with the conventional S10 plastination protocol, with the addition of a previous incision in the cuticle. In general, more studies are necessary to establish the changes in the standard S10 protocol necessary to carry out effective plastination of different parasite specimens, and to evaluate their anatomical structures for the best results.

References


Plastination Applied To The Conservation Of Cultural Heritage

ABSTRACT:
The archaeological excavation of a Phoenician shipwreck at Bajo de la Campana, San Javier, Murcia, Spain, was carried out from 2007 to 2011 under a collaboration agreement between the Institute of Nautical Archaeology of Texas A & M University, and the Ministry of Culture of Spain1, through the National Museum of Underwater Archaeology of Cartagena.
Throughout successive campaigns of systematic excavation, the archaeologists documented and raised an extraordinary cargo of a Phoenician wreck dated between 7th – 6th centuries BC. Among the raw materials it carried there was a magnificent set of 53 elephant tusks, and fragments of elephant tusks, some of them with inscriptions.
The uniqueness of this archaeological find is due to it being one of the few known examples of Phoenician navigation in the Mediterranean Sea, and the first time a shipment carrying ivory as raw material has been documented in Spain.
With this work, we present the results of the plastination process carried out on waterlogged archaeological remains, as an alternative to traditional preservation procedures on items of cultural heritage, which have proved to be ineffective due to the extreme density of ivory.
We tested the Biodur® S15 plastination technique at room temperature in successive phases, each step depending on the results obtained in the previous steps. Elephant tusk fragments were dehydrated with acetone, and subsequently impregnated with a mixture of polymer and catalyst, Biodur® S15 plus 1% S3. After impregnation, they were exposed to Biodur® S6 crosslinker, for the curing phase. The time taken for each step varied proportionally, according to the size of the ivory fragments.
The results obtained were satisfactory, both in dimensional stability and visual aspect, essential for the study and exhibition of these pieces that are part of our cultural heritage.
It should be noted that these optimum results have been maintained over time, two years since their processing, which validates this technique of plastination as a procedure for preserving waterlogged archaeological materials.

KEY WORDS: archaeology; conservation; ivory; plastination; Biodur©S15; waterlogged

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Introduction
The conservation of artifacts of our cultural heritage is based on a sound knowledge of the material's alteration factors, and the deterioration processes suffered during the burial period. In this case, it was necessary to carry out an initial characterization of archaeological waterlogged ivory, and compare it with samples of non-degraded ivory2. This enabled us to understand the structural components and diagenetic processes due to the marine environment.
Ivory is composed of a combination of organic and inorganic components, calcium hydroxyapatite and collagen type I, which are intimately related in a hierarchical way, from nano- to macro-scale, that gives
ivory its extraordinary physico-mechanical properties (Fig. 1).

The main diagenetic processes that the ivory had undergone during its burial period were partial dissolution of its structural components (mainly the organic fraction), and incorporation of exogenous elements that had replaced ions present in the hydroxyapatite structure, increasing the degree of crystallinity. In the case of iron, these had precipitated forming inclusions of pyrites that occupied fissures and interstices in the apatite matrix (Doménech-Carbó et al., 2016). As a result, we had elephant tusks that were highly degraded, and in which water had become part of the structure of the ivory, with a clear supporting function, so that its loss would cause internal stresses that would cause the structure of the ivory to collapse irreversibly. We carried out a study of waterlogged ivory degradation, and the damage suffered, on an archaeological ivory section from a Phoenician shipwreck (7th-6th centuries BC) at Bajo de la Campana³ (Figs. 2, 3), subjected to natural drying, without any conservation treatment. The section experienced a total weight loss of almost 44%. The most important changes occurred on the cross section, however, a few smaller changes were also observed in tangential and longitudinal sections, due to the anisotropy of the ivory (Fig. 4 i-iii).

³ Tusk fragment section, n° inv. SJBC_11_2471_3, dimensions and initial weight: Cross section: 5cm x 4 cm, thickness: 4.7 cm, waterlogged weight: 80.28 g.
As part of Spain’s cultural heritage, our priority was to preserve these items, in order to be able to access the historical and scientific information they hold. For this reason, it was necessary to apply a conservation procedure that allowed us to eliminate water, as well as guaranteeing dimensional stability of the artifacts.

The conservation of underwater cultural heritage begins with the implementation of a planned program of actions, from the initial planning of the archaeological intervention, taking into consideration human resources, materials, and infrastructures, both during the work at the site, and throughout the raising work, especially with the most fragile materials; and to then take them to the museum’s conservation laboratory, where the gradual transition from the marine environment to dry conditions will take place.

All artifacts that come to the conservation laboratory are individually inventoried and documented. In this way we record the initial conservation state and all necessary subsequent conservation treatments.

The conservation work began by removing soluble salts, by successive fresh water and demineralized water baths, to avoid further recrystallization and irreversible damage. The tusks were then prepared for drying, however, in the case of waterlogged materials, the degradation they would suffer made natural drying impossible. It was necessary to apply a conservation process that removes water, reinforces damaged structure, and guarantees dimensional stability of the artifact. The process must be compatible with the ivory and its exogenous components, (either from the marine environment, or neo-formed), that cannot be eliminated.

The Biodur® S10 and Biodur® S15 plastination procedures (von Hagens, 1988), originally developed and patented by Dr. Gunther von Hagens in the late 1970s for the preservation of organic specimens for anatomy teaching, provides characteristics that make it suitable for our purpose, such as chemical stability of its components, and good diffusion (Whalley, 1988; Wade and Lyons, 1995, 1999).

Ian Godfrey from The Western Australian Museum carried out plastination with Biodur® S10 on fragments of ivory from a Dutch merchant ship, the Vergulde Draeck (1656) obtaining optimal results at room temperature (Godfrey et al., 2012).

Other companies such as Silicone Inc., Corcoran Chemicals® or Dow Corning® have formulated very fluid
silicone polymers that can be used at room temperature, with slightly modified procedures: the North Carolina room-temperature plastination technique, and the Dow®/Corcoran technique (Raooof et al., 2007). The Archaeological Preservation Research Laboratory at Texas A&M University has worked with highly fluid polymers that can be applied at room temperature with almost no vacuum (silicone oil preservation) (Smith, 2003), which are more suitable conditions for conservation of archaeological artifacts. Wayne Smith has reported excellent results obtained with organic and inorganic materials from the wreck of La Belle (17th century), waterlogged ivory from the Tantura B shipwreck, (9th century), and from the wreck of the merchant ship the Vergulde Draeck (17th century).

We applied the Biodur® S15 plastination procedure at room temperature because we needed to dry and reinforce damaged archaeological ivory from Bajo de la Campana, and we lacked a detailed, easily reproducible plastination protocol. The most common protocol used in plastination laboratories is Biodur® S10 and Biodur® S15, although a slight modification of the latter technique was required for waterlogged archaeological ivory.

We proposed as the aims of this study, the development of a specific protocol for the Biodur® S15 plastination technique at room temperature, which would allow a better diffusion in an extremely dense material such as ivory, and enable not only the assessment its effectiveness on this type of material, but also the long-term stability of the treatment, through validation over time.

**Materials and Methods**

**Application of the plastination procedure, Biodur® S15 method, at room temperature**

**Experiments / trials**

The application of the Biodur® S15 plastination procedure was carried out in progressive steps, each depending on the results obtained in the previous steps.

Stage 1: To define a specific plastination protocol for an ivory specimen. It was performed on an elephant tusk section, the first time the procedure was applied to an archaeological artifact, specimen SJBC_11_2471_5a (Fig. 5). The specimen’s dimensions and weight before treatment were: cross section 5.5 cm X 4.2 cm, thickness 1.3 cm, waterlogged weight 49.01 g.

![Figure 5. Tusk section SJBC_11_2471_5a (Plastination Laboratory, Faculty of Veterinary Medicine, University of Murcia)](image)

Stage 2: Validate the protocol used in Stage 1 with an elephant tusk fragment, to verify the effectiveness of the procedure, and to establish if the shape and volume of the fragment will influence the behavior of the plastination process. The stability achieved in Stage 1, with the room temperature Biodur® S15 procedure, led us to investigate if equally good results could be achieved with a tusk fragment, that had not been sectioned. For this trial, we selected a tusk fragment with a preserved distal end, the proximal part having been mostly lost, specimen SJBC_10_2980 (Fig. 6). The specimen’s dimensions and weight before treatment were: straight line length between proximal and distal ends 22 cm, outer curvature 25 cm, maximum diameter 4.6 cm, waterlogged weight 373.61 g.

![Figure 6. Tusk fragment SJBC_11_2980 (MNAS photographic archive)](image)

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4 Dow Corning®: PR-10 hydroxyl-ended, functional polymer, CR-20 crosslinker (methyltrimethoxysilane/siloxane Q1-3563), CT-32, catalyst (dibutyltin diacetate)
Stage 3: The validated protocol was applied to a complete elephant tusk (SJBC_10_1926), for confirmation of the previous encouraging results, and the long-term study of the effectiveness of the treatment. The good results achieved on tusk section SJBC_11_2471_5a and on tusk fragment SJBC_11_2980, encouraged us to apply the S15 plastination procedure to a complete elephant tusk, SJBC_10_1926 (Fig. 7). The tusk dimensions were: straight line length between proximal and distal ends 72 cm, outer curvature 100 cm, maximum diameter 8.1 cm, waterlogged weight 4.850 kg.

Figure 7. Complete elephant tusk SJBC_10_1926 (MNAMES photographic archive)

The phases of the Biodur® S15 procedure at room temperature were, as in the standard protocol, dehydration, impregnation, and curing. Specimens were dehydrated in successive acetone baths, giving final acetone concentrations from 98.5% to 100%. Dehydration was carried out in a refrigerated chamber, at +5 °C, to avoid contraction and tension forces.

Impregnation was carried out at room temperature (18-20 °C) in a vacuum chamber, with the impregnation mixture of silicone polymer Biodur® S15 mixed with the chain-extender S3 (1%). The impregnation chamber pressure during impregnation was gradually decreased from 760 mm Hg to 3 mm Hg. Continuous bubbling was observed, as a consequence of acetone exit, and the progressive incorporation of polymer into the specimens. Impregnation was considered finished when no further bubbles appeared on the surface of the silicone bath. The vacuum was released, and the tusks remained in the impregnation bath overnight. The specimens were then taken out of the bath, and excess polymer was removed with cellulose tissue paper. Subsequently, the specimens were placed in a curing chamber where cross-linker (Biodur® S6) was vaporized to saturate the interior and cause curing to occur.

The chemicals used in this procedure were acetone, Biodur® S15 polymer, Biodur® S3 catalyst, and Biodur® S6 curing agent (von Hagens et al., 1988; Henry et al., 1997; Latorre et al., 2001; Smolak et al., 2005; Ottone et al., 1988).

All the archaeological ivory sections, tusk fragments and complete elephant tusks were documented before and after plastination, photographed, measured, and weighed, to record any changes that occurred during processing.

Results

Table 1 shows the time taken for the three plastination stages for each specimen. Table 2 shows the weight of the specimens, from the initial to the final weight, and percentage weight loss.

Specimen 1: section nº 5a from tusk fragment SJBC_11_2471_5a (Figs. 8-11).

<table>
<thead>
<tr>
<th>Inventory number</th>
<th>DEHYDRATION</th>
<th>IMPREGNATION</th>
<th>CURING</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acetone 5 deg C.</td>
<td>S15+S1 Room temp.</td>
<td>S6 Room temp.</td>
</tr>
<tr>
<td>SJBC_11_2471_5a</td>
<td>3 days</td>
<td>3 days</td>
<td>3 days</td>
</tr>
<tr>
<td>(piece 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SJBC_11_2980</td>
<td>27 days</td>
<td>14 days</td>
<td>12 days</td>
</tr>
<tr>
<td>(piece 2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SJBC_10_1926</td>
<td>27 days</td>
<td>30 days</td>
<td>12 days</td>
</tr>
<tr>
<td>(piece 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Time of Biodur® S15 plastination at room temperature of ivory pieces 1, 2 and 3
The total plastination time for this specimen was 9 days (Table 1). After dehydration with acetone, an incomplete crack was observed. During impregnation, no incidents were detected, and the crack did not increase. When the tusk section was exposed to cross-linker, it began to change color until the drying was complete. The final appearance was very similar to dry ivory; no glossy deposits of polymer were seen on the surface or interstices (Fig. 12).

After curing, the weight of the tusk section was monitored, and it was found to decrease from 46.72 g to 44.01 g at three months. The fissure remained stable, it did not progress, and no new cracks appeared. The difference between initial and final weight was 5.55 g, 11.32% of its initial weight (Table 2).
Table 2. Weight of ivory pieces before and after plastination, and percentage weight loss

<table>
<thead>
<tr>
<th>Inventory number</th>
<th>Waterlogged initial weight (grams)</th>
<th>Final weight After treatment (2014) (grams)</th>
<th>Final weight 3 months after treatment (grams)</th>
<th>Final weight 3 years after treatment (grams)</th>
<th>Weight lost (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SJBC_11_2471_5a (Piece 1)</td>
<td>49.01</td>
<td>46.72</td>
<td>44.01</td>
<td>43.46</td>
<td>11.33</td>
</tr>
<tr>
<td>SJBC_11_2980 (Piece 2)</td>
<td>373.61</td>
<td>370.70</td>
<td>365.75</td>
<td>365.96</td>
<td>2.05</td>
</tr>
<tr>
<td>SJBC_10_1926 (Piece 3)</td>
<td>4850.0</td>
<td>4445.0</td>
<td>4445.0</td>
<td>4443.28</td>
<td>8.61</td>
</tr>
</tbody>
</table>

Specimen 2: Tusk fragment SJBC_11_2980 (Figs. 13-15)

Figure 13. Tusk fragment SJBC_11_2980 plastinated (MNAS photographic archive)

Figure 14. Mechanical removal of polymer on tusk fragment SJBC_11_2980. The center line divides the left side, still with polymer, and the right side were we have removed both polymer and the original surface (MNAS photographic archive)

Figure 15. Tusk fragment SJBC_11_2980 plastinated, after removal of surface polymer layer. (MNAS photographic archive)
The complete process for this specimen took 53 days (Table 1). During dehydration, no fissure, crack, or detachment was observed, and the tusk fragment remained stable. After impregnation, the excess polymer was not removed; on the contrary, more impregnation mixture (S15 + S3 1%) was applied to the surface, to determine if leaving this thick layer on the specimen was more effective in preventing possible loss or detachment of cementum or dentin. It also allowed us to investigate how to remove excess surface polymer during curing, and if, in doing this, any loss of material occurred (Fig. 13).

The final weight of the plastinated tusk fragment was 365.96 g, weight loss 2.05% (Table 2).

Removal of the curing polymer from the surface of tusk fragment SJBC_11_2980 was carried out using mechanical procedures. We used a scalpel, by scraping the superficial layer of silicone, and, subsequently, rubbing the residual polymer with a swab (Fig. 14). It was even possible to remove this layer by using a swab alone, by scraping and rubbing with the swab, because the cotton adhered to the silicone and removed it perfectly. It took a little more time but was very controllable and effective. The original surface of the tusk fragment was preserved, so that the original rough appearance can be seen. No detachment of the tusk surface occurred, and the fragment remained stable (Fig. 15).

**Specimen 3: Complete elephant tusk SJBC_10_1926 (Figs. 16-19)**

The complete plastination procedure took 69 days. During dehydration and impregnation (Figs. 16-18) no fissures, cracks or detachment appeared; the tusk remained stable.

![Figure 16. SJBC_10_1926 Complete elephant tusk (MNAS photographic archive)](image)

![Figure 17. During impregnation of complete elephant tusk SJBC_10_1926 (Laboratory of Plastination, Faculty of Veterinary Medicine, University of Murcia)](image)

![Figure 18. Complete elephant tusk SJBC_10_1926 plastined (MNAS photographic archive)](image)

![Figure 19. After mechanical removal of the polymer on surface of complete elephant tusk SJBC_10_1926 (MNAS photographic archive)](image)
stable. After impregnation, the excess polymer was removed from the surface with cellulose tissue paper. This caused local detachments of cementum. A small fragment of the proximal end that had a micro-crack prior to treatment also became detached (Fig. 19). It appeared that leaving a thick layer of excess polymer protected the tusk surface. The surface-cured polymer was cleaned by mechanical means. By rubbing with a swab, the polymer was removed smoothly (Fig. 19). No new cracks have been detected, nor has there been any progression in existing defects. The final weight was 44432.80 g; weight loss was 8.61% (Table 2).

Discussion

Waterlogged elephant tusks recovered and documented from archaeological sites around the world are very scarce, which is why little is known about their conservation.

Our objective was conservation of waterlogged elephant tusks from the Phoenician Bajo de la Campana shipwreck, and, in doing so, to enhance our knowledge of the plastination procedure and its application to conservation of cultural heritage. We have, for the first time, documented the Biodur®S15 plastination technique on waterlogged archaeological ivory, and how to remove the excess cured polymer from its surface. Our results provide an easy-to-reproduce protocol for using the Biodur® S15 plastination technique at room temperature.

In previous conservation work on waterlogged archaeological ivory, satisfactory results were obtained with Biodur®S10 and the silicone oil /siloxane method, respectively (Godfrey et al 2012; Smith 2003). Smith used a variation in the standard method, which was impregnation with a mixture of polymer and cross-linker with light vacuum, and curing with catalyst. Godfrey used Biodur® S10 and siloxane Q1-3563 containing MTMS (9%) (Godfrey et al., 2012), while Smith used SFD-1 (Dow Corning® PR-10) silicone oil containing 5% methyltrimethoxysilane (Dow Corning® CR-20, crosslinker) and dibutylin diacetate (Dow Corning® CT-32, catalyst) on waterlogged archaeological ivory from the Vergulde Draeck shipwreck (17th C) (Smith, 2003). In both cases, the steps used were dehydration with alcohol or acetone, impregnation at room temperature, and curing; this is the basis of all room-temperature protocols, in order to avoid the silicone becoming viscous too quickly from the effects of the catalyst or chain elongator (Ludwikowski et al., 2001; Raoof, 2001).

In our opinion, the critical point of this plastination procedure was to be able to preserve the integrity of the specimen, particularly the most fragile fragments on surface, avoiding any becoming detached. The fissures and detachment that we have documented occurred because we worked with highly degraded material, however, they were extremely useful to improve the procedure. The results obtained in our study show that the Biodur® S15 plastination technique at room temperature, was successfully applied to the Bajo de la Campana ivory.

Dehydration was the stage where we observed some changes, a small crack appeared in specimen 1 (Fig. 8) which seemed to be related to the size of tusk section and the length of time spent in this phase. In addition to this, water plays an important role as a cohesive element in organic and mixed waterlogged archaeological materials.

Other authors who have worked with waterlogged ivory, and other archaeological materials such as waterlogged wood or leather, (De La Cruz Baltazar, 1996; De La Cruz Baltazar et al., 1999; Bouzas et al., 2008), have not reported any limitations during this phase.

The impregnation phase was carried out without problems. The mixture of silicone polymer and catalyst was perfectly incorporated. Bubbling indicated the evaporation of acetone until it ceased; the larger the piece, the longer this phase takes. The end of the process was reached at pressures between 5 mm-2 mm Hg; this did not cause any damage to the archaeological ivory.

An important disadvantage of silicone oil preservation is the partial loss, by evaporation, of cross-linker at pressures below 10 mm Hg during the impregnation phase. This means that there is no control over the percentage of cross-linker remaining in the impregnation mixture.

Tests performed on Specimen 2 showed that it was necessary to keep a layer of silicone on the surface as a stabilizing element for the more fragile lamellae. Subsequently, as we have explained above, it is then necessary to remove part of the protective layer of silicone mechanically, in order to achieve a natural final appearance.

The successful control of both weight loss and shrinkage of the Bajo de la Campana's ivory pieces shows that a significant dimensional stability can be achieved, a crucial requirement for underwater archaeological heritage conservation. On the one hand, the polymer becomes a
supporting element of the damaged structure, replacing the structural components lost or transformed during the immersion period; and, on the other hand, it stabilizes even the most unstable incorporated and neo-formed compounds, such as pyrite (Doménech et al., 2016). The maximum weight loss obtained with the Biodur® S15 plastination technique at room temperature was 11%, however, previous assays in our lab with the classical protocol of monitored desiccation showed a weight loss greater than 40%.

Godfrey et al., (2012) analyzed plastinated ivory fragments ten years after treatment, and reported no apparent changes. They emphasized the high degree of penetration, homogeneous diffusion, and structural reinforcement of ivory specimens with diverse initial states of conservation, both slightly degraded and very degraded. However, these authors do not comment on long-term weight loss.

Our results, two years after treatment, validate Biodur® S15 plastination at room temperature as a conservation method, accomplishing an optimal final appearance. The plastinated ivory has a natural color, and the texture, (whether the excess polymer is removed with cellulose paper after impregnation, or mechanically removed after curing), is practically same as the original.

This plastination procedure achieves the main objectives of water removal and dimensional stability. Plastination is also reversible, a preferred, but not essential, quality in conservation of cultural heritage. It is important to have the possibility of reversing the procedure, in case it causes problems in the future. The process of de-plastination of plastinated ivory could be an area for future study, since there are currently no references to this in the literature (Walker et al., 1988, De La Cruz Baltazar et al., 1999).

In conclusion, plastination can be an effective conservation procedure for waterlogged organic and mixed materials, although we will continue to monitor its long-term outcomes, and continue our research into the conservation of waterlogged ivory.

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Conservation of Cultural Heritage


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Whalley A. 1988: Practical applications of plastination in archeology. 4th Int Conf Plast, Macon, GA, USA.
The Challenges of Plastinating a Blue Whale (Balaenoptera musculus) Heart

ABSTRACT:
A tragic mortality event for the Northwestern Atlantic population of blue whales occurred in March 2014. Two blue whale skeletons were salvaged by the Royal Ontario Museum, which also afforded the opportunity to salvage and preserve a blue whale heart. The technical challenges to preserve this heart by plastination are presented. The resultant plastinated mature blue whale heart demonstrated some variation from typical terrestrial mammalian hearts. However, this heart confirmed anatomical details of other cetacea and marine mammal species. The plastinated blue whale heart promises to be an enduring asset of tremendous scientific, educational and artistic value.

KEY WORDS: blue whale, Balaenoptera musculus, heart, plastination, preservation, massive

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Introduction
Plastination has become the gold standard for preservation of anatomical specimens by replacing tissue fluid with a curable polymer. The longevity of plastinates is advantageous for preservation biological tissue, but especially rare or unique specimens of inherent scientific interest (von Hagens, 1985; von Hagens et al., 1987). Such is the case with the world’s first plastination of a blue whale heart.

Whales are iconic biological organisms, notable for their extraordinary anatomical and physiological adaptations to their obligate marine existence. The challenges and constraints imposed by this aquatic lifestyle have likely affected organ development and physiology. The blue whale is the largest of cetacean species and is the most massive animal throughout history (Small, 1971; Reeves et al., 1998; Sears and Perrin, 2018) and thus has captured scientific interest.

The blue whales, Balaenoptera musculus, can be divided into at least three subspecies and several unique populations, such as the blue whales of the North Atlantic Ocean. The excess harvest of most of the large whale species during the commercial whaling era resulted in a moratorium by the International Whaling Commission which banned commercial hunting of blue whales in the North Atlantic in 1955. Full protection of this species was extended globally by 1966 (Small, 1971; Sears and Calambokidis, 2002), along with many other whale species since then. To date, the blue whale remains critically endangered, and numbers of the Northwestern Atlantic blue whale population remain low (Sears and Calambokidis, 2002; Reilly et al., 2008; Beauchamp et al., 2009).

Limited anatomical data for larger whales has been derived from commercial whaling or from mortality events and their associated necropsies. Deceased blue whales generally sink and rarely beach (Tønnessen and Johnsen, 1982), therefore, anatomical studies are few.
In March 2014, Northwestern Atlantic blue whales experienced a tragic loss. Nine whales were found dead in the ice pack near the Cabot Strait in the Gulf of Saint Lawrence, Canada. This loss was significant, since population size is estimated only in the hundreds for this unique Northwestern Atlantic population (Sears and Calambokidis, 2002; Reilly et al., 2008; Beauchamp et al., 2009). In the spring, two dead whales drifted to the southwestern coast of Newfoundland, Canada (Allen, 2014). The Royal Ontario Museum (ROM) obtained permits from the Canadian Department of Fisheries and Oceans to salvage skeletons from these two carcasses (permit No. NLSAR-003-14). The larger, 24.5 metre beached female was processed at Woody’s Point for a complete skeleton. The smaller, 23 metre (76 foot), mature female, processed at Rocky Harbour (Fig. 1), was partially submerged in cold water until necropsy, possibly retarding autolysis to some degree. Thus, the rare opportunity arose to salvage an intact blue whale heart.

**Materials and Methods:**

**Specimen removal**

The heart of the 23 m (76 feet) mature blue whale (Fig. 1) (ROM number-125066) was collected. The vertebrae and ribs have osteoarthritic lesions which indicate an older specimen. Flensing (removal of soft tissue) of the whale carcass began caudally and proceeded cranially. Thus, abdominal cavity viscera were removed prior to thoracic cavity viscera. The heart was exposed after removal of the thoracic wall muscles (Fig. 2). With the whale in dorsal recumbency, visualization of the heart’s dorsal attachment was difficult. The great vessels (aorta and cranial and caudal vena cavae) were identified and blindly transected for removal of the heart and lungs together. However, this unit was too large to maneuver through the intercostal space. It was necessary to separate the lungs from the heart by cutting the pulmonary vessels. Detached from the carcass and lungs, the heart was pushed out of the thoracic cavity (Fig. 2), into a nylon bag, and lifted away by a front-end loader. The heart was stored in a refrigeration truck at -1°C to -2°C and transported with the skeletons to Research Casting International, Trenton, Ontario. The heart was stored at -20°C for over a year, until personnel could be assembled to proceed with cleaning, dilation and formalin-fixation. The heart weighed 175 Kg (386 pounds). An appropriate stainless tank [1.6 m x 1.75 m x 1.25 m (3,500 L)] was fabricated for thawing, dilation, fixation and storage.

**Figure 1.** Beached, 23 m blue whale: dorsal recumbency.

**Figure 2.** Blue whale heart: at intercostal space.

**Specimen preparation**

In June 2015, the heart was thawed by running tap water over the heart and finally immersion in a tank with running water for 24 hours. After partial thawing, water was directed into the chambers through the transected vascular ports, to complete thawing and flush the chambers of debris (Fig. 3). Superficial landmarks along with the great vessels were identified for heart orientation. Extraneous tissue was removed. Limited morphological measurements were taken and recorded. To flush and dilate each side of the heart (Tiedemann and von Hagens, 1982; Oostrom, 1987a; Henry et al., 1997), a cannula was secured into both atria via the cranial vena cava and a pulmonary vein. All remaining ports were occluded by securing an appropriate diameter stopper (i.e. drink bottles to 20 L buckets) into each vessel/port. Small rents were sutured. After closure of cardiac ports, tap water was directed into the submerged heart for dilation (24 h). Only partial dilation was achieved due to leakage through autolytic tissue and small lacerations, small ports and low water pressure.
For fixation, the tank and heart were transferred via a lift (Fig. 4) to a paint room with dedicated ventilation. Twenty-percent formalin solution was prepared and used to dilate the heart and displace the remaining water in the heart. 200 L of 100% formalin were diluted to ~20% to submerge and keep the heart dilating (Oostrom, 1987b). Filling, dilation and maintenance of partial dilation of right and left hearts was accomplished using two submersible pumps (1/5 HP; 1700 GPH) (Fig. 5). The buoyant heart was covered with formalin-soaked towels and a smaller submersible pump (170 GPH) was used to pump fixative over the towels to keep the surface of the heart moist. Maximal dilation of the heart could not be maintained due to fixative leakage. For long-term, the final concentration of formalin was reduced to 15% by addition of tap water. The fixed and partially dilated heart was stored in 2300 L of 15% fixative for 5 months. A total of 600 L of 100% (37% stock solution) formaldehyde was used to provide the 20% and 15% formalin aliquots needed for fixation and storage. For the plastination venue, it was decided to utilize a working facility that was equipped to handle very large specimens and large volumes of acetone rather than to develop such a large and expensive lab for likely limited use. Gubener Plastinate, Gmbh, Germany was suitable, and was chosen.

Packaging and shipping

Customs offices at each border crossing were contacted in advance, to confirm customs documentation and requirements for both countries. Formalin-fixed specimen shipment [International Air Transport Association (IATA...
guidelines), endangered species transport, and agricultural and zoosanitation shipment requirements all had to be reconciled (CITES export permit #15CA02942/CWHQ, CITES import permit # E-04016/15). To satisfy zoosanitation considerations, the findings of Rutala and co-workers (2008) for efficacy of formaldehyde treatment in neutralizing microorganisms, were followed.

The heart was drained of fluid by removal of the three largest vascular port stoppers (cavae and aorta). A nylon dumpster bag was placed under the heart to raise it from the tank and the fixative solution. The tank was drained and cleaned. A wrapping protocol to comply with the IATA guidelines, included: 3 layers of poly sheet, 2-4 mm, thinnest layer next to the heart. Cellulose material (void-fill absorbent) was placed on each plastic layer. For wrapping, the heart was placed on top of this sandwich of plastic and fill. The void-fill next to the heart was saturated with water. The packaged heart was lifted back into the steel tank, which was lined with polyurethane foam sheets. Sacks of foam peanuts were placed in each corner, to create a void into which the wrapped heart would be cradled. The heart was covered with polyurethane foam and the tank sealed with silicone. Kuehn + Nagel Ltd. transported the packaged heart from Trenton, Ontario to Guben, Germany.

**Vascular injection**

The heart arrived in Guben, November 27, 2015 and was unpacked and inspected. The heart was dorso-ventrally flattened and had a typical formalin-fixed beige color. Multiple small cuts and imprints from packing material and stoppers were observed along with areas of arrested decomposition. The heart was transferred to a steel tank (2 m x 2 m x 1.5 m) filled with water (Fig. 4). All stoppers were removed, and the heart chambers were flushed with tap water for 24 hours.

The S14/S1 (silicone polymer [S10]/hardener [S30]) protocol was used for vascular injection (Biodur products, 2006; Coman et al., 2014). The heart was submerged in water for injection which allowed silicone leakage to float to the surface and allow visualization of defects in the vasculature. Red S14/S1-mix (80 L) was injected into the cannulated coronary arteries and 40 L of blue silicone-mix [S10, S1 (3%), AC52 (2%)] into the great cardiac vein/coronary sinus. Polymer leakage (10 L red and 20 L blue) was significant from damaged vessels (Fig. 6). After vascular injection had cured, the heart was bleached for 10 days by immersion into 5,000 L of 3% hydrogen peroxide to facilitate recolouring.

**Dehydration**

Gubener Plastinate GmbH’s largest vacuum chamber (4 m x 3 m x 2.2 m) was selected for two major processes of plastination: 1) dehydration with defatting and 2) impregnation (deJong and Henry, 2007). The vacuum chamber was located outdoors under cover. Since cold acetone is critical to the success of dehydration and forced impregnation (von Hagens et al., 1987), the chamber’s freezer unit afforded the required temperature of -25°C for both dehydration and impregnation. Dehydration was in a stainless steel tank nested inside the vacuum chamber. In order to stabilize and retain the natural shape of the heart during the dehydration process, an acetone resistant, low temperature resilient 1 cm thick nylon mesh was inserted as needed inside the heart and great vessels. The first phase of dehydration required five changes of cold acetone (-25°C) over 42 days. The second phase of dehydration, de-fatting in ambient temperature acetone (Tiedemann and Ivic-Matijas, 1988; Brown et al., 2002), was carried out over 4 months during the spring and summer months in Guben, Germany. To obtain ambient temperature acetone, the vacuum chamber cooling unit was shut off during this time. The dehydration process was completed on August 4, 2016. A total of 22,000 L of acetone was used during the entire dehydration process. The heart was raised and drained of excess acetone in final preparation for the next step, forced impregnation.

**Impregnation**

On August 4, 2016, following dehydration and draining excess acetone, the heart was submerged in 5000 L of cold (-25°C) S10/S3, silicone-catalyst mixture (100:1 ratio). Biodur AC50 red color paste was mixed into the polymer to tint the impregnation-mix as desired. Before applying vacuum, the heart was allowed to equilibrate for 2 days in the impregnation-mix. As an optional time saving procedure, residual acetone was removed from the surface of the impregnation-mix after submersion of the
heart and before application of vacuum. The initial pressure applied to the vacuum chamber was lowered from ambient to 150 mbar (112 mmHg). Pressure was decreased slower after 150 mbar, until near the vapor pressure of acetone (21 mbar [~15.8 mm Hg] at -25°C) when acetone began to be extracted. Vaporization of acetone was visually monitored by bubble formation. Acetone extraction/vaporization was active for 80 days. The final pressure was 5 mbar (3.75 mm Hg) which was maintained until bubbles nearly diminished. When impregnation was complete, the vacuum was released and the heart was brought back to ambient pressure by mid-December, 2016 (Henry and Nel, 1993; de Jong and Henry, 2007). The heart was removed from the cold polymer-mix to allow surface polymer to drain freely at ambient temperature. The nylon mesh structural support was removed from the interior of the impregnated heart. The heart remained at ambient temperature for several weeks to allow for draining of excess polymer, positioning and dissection.

**Positioning and dissection**

Following impregnation, the malleable tissue of the heart afforded further anatomical positioning and dissection.

**Positioning:** The left ventricle was contoured to near diastolic conformation by using a steel armature inside the ventricle, which also served as a permanent internal scaffold. A 6 cm tube was inserted from the apex, toward and into the aorta to the origin of the brachiocephalic trunk to support the heart in a vertical orientation. This post was retained for connection to the external floor stand.

**Dissection:** The coronary groove was dissected to demonstrate the branches of the great vessels and their distribution. A 25 cm window was cut into the auricular surface of the right ventricle to expose the right atrio-ventricular (A-V) valve. Extraneous fascia was removed from the basal structures of the heart (great vessels and pulmonary vessels).

**Curing:** In preparation for curing, additional dilation of the chambers was completed by applying tension to the walls in needed directions by means of hooks and ratchets. Once in final position, the impregnated silicone in the heart was hardened by exposure to S6 vapors. Curing at ambient temperature continued for ten days. To enhance the color of the heart for final display, and to stabilize surface detail, a mixture of S10/S3 and red color (AC50), tinted to a desired color, was brushed onto the surface of the heart. After the desired S10+S3 color-mix was created and brushed onto the heart surface, it was cured overnight with S6 vapor.

**Results**

The autolytic heart (Fig. 2) of a decomposing 32 m, adult, female North Atlantic blue whale (*Balaenoptera m. musculus*) was successfully preserved by fixation and the Silicone S10 Standard (Cold) Procedure cold silicone plastination (Fig. 7). To appreciate the more natural aesthetics of the heart, after the first S6 cure, a colored impregnation-mix was applied to the surface, creating a pinkish hue to the once achromic formalin-fixed heart. The color was successfully set during the second and final S6 curing process. Upon completion of the hardening process, the thick ventricular walls, aorta and pulmonary trunk were not flexible. The thinner structures: vena cavae, pulmonary vessels and atria, maintained some flexibility. The shape and conformation of the heart was preserved in a near diastolic state. External gross structures were well-preserved and observed. The internal morphology that could be seen from the limited visual vantage was also preserved. Coronary vasculature
was enhanced by injecting with colored silicone polymer-mix. The heart revealed a dorso-ventral flattening, a bifid apex, and a well-defined right/left orientation of the chambers, especially on the dorsal/atrial aspect of the heart.

The final dimensions of the plastinated heart were: width: 96.3 cm and length: 106.2 cm.

Figure 8. Royal Ontario Museum Exhibit: Dilated, dissected, cured, plastinated blue whale heart. Auricular view. RV - right ventricle with port. Bar is 13 cm.

Discussion

The plastinated blue whale heart is an asset to ROM's research collection and a significant element of the ROM's travelling exhibition, “Out of the Depths: The blue whale story”. As expected, the plastinated heart is dry, non-toxic, and requires no additional preservation medium, which adds to its significance in the museum's scientific collection. The morphology of the salvaged, autolytic blue whale heart (Fig. 2) differed from that of the typical terrestrial mammalian heart morphology: dorso-ventral flattening, bifid apex, and right/left orientation of the chambers.

The major hurdle during preservation was the heart's large size, 175 Kg (386 pounds), along with its wet, slippery nature. Therefore, a lift and netting (Fig. 4), were needed to move the heart through each stage of the plastination process. It was difficult to turn the specimen, even when floating, to facilitate inspection of any area except that which was on the upper surface. The heart was kept moist and formalin conserved by pumping fixative over the towels covering the heart. This ensured reasonable fixation, and retarded autolysis. The autolytic heart had many open vessels which allowed excessive leakage of colored silicone while injecting the coronary vessels. The heart was submerged in water to inspect for leakage, and to keep the large amounts of leaked polymer from coating the specimen (Fig. 6). This allowed the less dense, leaked polymer to float to the surface. A heavy-duty internal support was designed to support and display the nearly 400 lb heart, and to aid in dilation.

It is interesting to note that the general principals of plastination of this large heart: specimen preparation, dehydration/defatting, impregnation and curing, were basically similar to principals for smaller more routine specimens (von Hagens, 1979a, 1979b; Oostrom, 1987a, 1987b; Tiedemann and Ivic-Matijas, 1988; Henry and Nel, 1993; Henry et al., 1997; deJong and Henry, 2007). These previous plastinated specimens have stood the test of time. Impregnation, probably one of the more critical aspects of the plastination procedure, was carried out at the same pressure, when temperature was considered, for both large and small specimens. The one prolonged variable, time of impregnation, was twice as long as for most smaller specimens. The limitation presented by the whale heart was basically volume: a matter of handling and moving the large, slippery specimen.

The unique occasion to plastinate a blue whale heart, likely the largest animal to have existed, provides an opportunity to study in detail an organ subject to several important physiological constraints. Only through an international scientific collaboration between two plastination laboratories, Lincoln Memorial University, Harrogate, TN and Gubener Plastinate GmbH, Guben, Germany, and the Royal Ontario Museum, Toronto, Canada, was this project possible. The methods described here transformed a severely-autolytic, rare specimen, into a durable specimen, while retaining accurate morphology. The project afforded insights into the challenges of plastinating very large organs, by building upon prior methodologies suitable for smaller organs (Tiedemann and von Hagens, 1982; Oostrom
This project also demonstrates that successful plastination of suboptimal material that has already undergone substantial autolysis and degradation (Fig. 2) is possible. This information should open the door and increase the potential for future recovery and preservation of rare material.

The blue whale is an iconic emblem of conservation. Opportunities to study the anatomy of this critically endangered species are rare, and every effort to preserve important anatomical material in an enduring fashion should be pursued. Preservation of the blue whale heart by plastination through cold temperature forced impregnation ensures this rare specimen will be available for study and education for a long time.

References


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Figures 1, 5, 7, Courtesy of Lincoln Memorial University, College of Veterinary Medicine, ©LMUcvm

Figures 2, 3, 8, Courtesy of the Royal Ontario Museum, ©ROM

Figures 4, 6, Courtesy of von Hagens Plastination, ©vHP
Rehabilitation of Plastinated Anatomical Prosections Using Silicone Adhesive and Pre-Cured S10/S3-Impregnated Fascia and Muscle

ABSTRACT:
Continual heavy use of plastinated prosections in the anatomy laboratory increases the risk of damage to specimens and diminishes their educational value over time. The plastinated anatomical teaching collection at New York University College of Dentistry was procured in 2005 for the gross anatomy course. The utility of our collection is dependent upon periodic maintenance and restoration of structures, which have been damaged due to prolonged handling. Four plastinated prosections with damaged or misplaced structures were chosen to demonstrate tissue-specific restorative techniques for artery, nerve and muscle. This article describes restorative procedures involving the use of household silicone adhesive followed by integrating pre-cured, S10/S3-impregnated fascia and muscle to recover anatomical appearances. Detached muscle was slow-cured before it was reattached with silicone and exposed to S6 hardener curing fluid by injection. Photographs were taken of plastinates during and after the repair process to document damage and restoration. Results show that each procedure restored the normal anatomical appearance of all damaged structures. While silicone adhesive works well to mend broken structures, the integration of pre-cured, impregnated fascia and muscle helped to strengthen damaged structures and conceal the damage. Periodic examination and maintenance of wear and tear on plastinated prosections will allow them to resume their previous anatomical appearance, and extend their usefulness and educational value.

KEY WORDS: anatomy; dental education; fascia; plastinated prosections; repair; restoration

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Introduction
Plastination, developed by Dr. Gunther von Hagens, preserves biological tissues in curable polymers (von Hagens, 1987). Plastinates have transformed the study of anatomy in the laboratory because of their dry, odorless, and non-toxic qualities. In 2005, human plastinated prosections were purchased from von Hagens Plastination and are used in the dental curriculum of the Department of Basic Science and Craniofacial Biology, New York University College of Dentistry (NYU Dentistry) (Baker, et al. 2013). All plastinated materials came from cadavers donated to The Body Donation Program of the Institute of Plastination in Heidelberg, Germany. Our plastinates were produced at the Gubener Plastinate GmbH (Plastinarium) using the BiodurTM S10/cold temperature plastination technique, considered to be the gold standard for plastination for over 35 years (de Jong & Henry, 2007). Our plastinates exhibit a high degree of anatomical specificity and are handled by 380 new students and faculty members each year.

The extensive use of plastinated prosections in the classroom increases the risk of damage from wear and tear and will, if neglected, accumulate over time. Significant damage diminishes the educational utility and potential useful shelf life of plastinated materials. While plastination technology renders anatomical structures durable, no material is indestructible. Neurovascular structures are damaged most often, but muscle and bone...
are also susceptible to damage. The utility of our teaching collection for the department’s anatomy course is therefore dependent upon the proper maintenance and effective restoration of plastinated prosections. Minor damage from wear and tear, such as torn arteries, can be repaired with the use of a common silicone adhesive (Raoof, 2014). However, even when mended this way, such structures often leave visible traces of previous damage and repair, which can compromise aesthetic integrity and, potentially, anatomical clarity. Damage that is more serious may be provisionally mended with silicone, but the original integrity of the damaged tissue may not be restored with the use of silicone adhesive alone. Fast-acting cyanoacrylates (e.g. super-glue) should be avoided when mending plastinated structures. Due to the brittleness of these adhesives, structures mended with cyanoacrylates become susceptible to fracturing and splintering, and will further degrade plastinated tissue. We have seen this occur to structures of older specimens repaired this way in our collection.

While silicone adhesive alone can resolve most minor damage, it often fails to restore the original appearance of larger structures, as it leaves behind evidence of damage and repair. This paper describes tissue-specific restorative methods, where plastinated muscle and neurovascular structures were reconnected with silicone adhesive, and whose anatomical appearances were recovered by integrating pre-cured, S10/S3 impregnated fascia and/or muscle onto the damaged surface. Completely detached muscle was also fully replaced with pre-cured, S10/S3 polymer-impregnated muscle tissue, which was then cured. The purpose of this paper is to provide a guide for other departments that have purchased S10 plastinated prosections for their medical and dental curricula, or that plastinate their own organs and cadaveric body parts for student use according to the S10/cold temperature technique.

Materials and Methods

Specimens
Four plastinated prosections with damaged or misplaced structures were chosen to demonstrate tissue-specific restorative techniques for artery, nerve and muscle: two bisected heads: a. deep, and b. superficial prosections of head and neck (Figs. 1-2a); one deep prosection of the infratemporal fossa showing a wrongly positioned auriculotemporal nerve (Fig. 3), and one deep prosection of the back region showing the infraspinous fossa with a detached infraspinatus muscle (Fig. 4a).

Restoration materials
A self-curing silicone adhesive (GE Silicone II* Household Glue) was used to mend and re-position detached structures. Pre-cured, S10/S3-impregnated fascia and/or muscle were integrated into the restoration area to strengthen damaged structures, replace detached muscle, and restore anatomical appearances. Impregnated repair tissues (fascia and muscle) were provided by the Plastinarium in Guben, Germany. Biodur® S6 hardener was used as a cross-linking agent to cure the S10/S3 impregnation mix.

Restoration equipment
Various operative instruments commonly used in dentistry were used for the application of silicone adhesive and restorative tissues during the restoration process. These instruments include dental Woodsons, surgical curettes, scalpels, forceps, and bone cutters. Other implements include insect pins of various sizes, plastic wrap, 1 ml and 24 ml syringes, and a small paintbrush.

Procedures
Most broken structures were re-connected with silicone adhesive at room temperature and blended with pre-cured, S10/S3-impregnated fascia. Structures were anatomically positioned with insect needles to facilitate the application of all restoration materials. Insect pins were removed after the silicone adhesive had cured for 12 hours. Pre-cured tissues were subsequently cured by injection with Biodur® S6 Hardener. All plastinated prosections were photographed before, during, and after the repair process, to document the damage, each stage of the restoration procedure, and the results.

Figure 1: A. Deep prosection of head and neck with severed carotid arteries. B. Insect pin inside lumen of arteries (arrows). C. Silicone adhesive spread around repair margin (arrows). D. S10/S3-impregnated pre-cured fascia ready for application over repair sites (arrows) (E, F, G) and blended around the arterial wall with silicone (H).
Insect pins were cut to size with bone cutters and placed within the lumina of both the internal and external carotid arteries to support reattachment. Insect pins were also inserted around the vessels to position them in their anatomical position. Silicone adhesive was applied to the broken ends of the vessels with a small paintbrush. The vessels were then reattached with the internal support of the insect pins. Pre-cured fascia was applied over the repair site, and unfurled and contoured around each vessel using the flat end of a Woodson. This was repeated until the fascia was fully blended around the arterial wall, and then 'painted' over with a light coating of silicone adhesive in order to camouflage the damage and restore the anatomical appearance.

The platysma was first realigned along the inferior border of the body of the mandible with insect pins, and then reattached with silicone adhesive. A portion of pre-cured, S10/S3-impregnated fascia was then flattened over a hard surface lined with cellophane wrap, and cut into small strips with a scalpel. Using forceps, the strips were then applied perpendicularly along the repair margin between the reattached platysma and the inferior border of the mandible. The strips of fascia were contoured into the grooves of the muscle with a Woodson in order to blend and integrate the fascia into the muscle, and restore normal anatomical appearance. Silicone adhesive was then applied over the repair site to further seal the fascia, and camouflage the damage.

A fractured area of the reattached platysma was repaired by integrating S10/S3-impregnated, pre-cured muscle, which was cut separately into strips with a scalpel. The strips of muscle were dipped into silicone adhesive, positioned over the fracture, and gently integrated into the host muscle until the fracture was covered. Additional strips of pre-cured muscle were applied perpendicularly in between the reattached platysma and the inferior border of the mandible, and then blended with silicone. This was repeated several times in order to reintegrate and reinforce the platysma onto the mandible. Light coats of silicone adhesive were also applied over the reattachment site to self-cure and add more protection over the integrated tissues.

Figure 2a: A. Superficial prosection of head and neck, with detached platysma. B. Muscle realigned to mandible with insect pins and reattached with silicone adhesive. C. S10/S3-impregnated pre-cured fascia strips. D. Pre-cured fascia applied along repair margin. E. Fascia blended with silicone. F. Ruptured muscle beneath mental eminence repaired in Figure 2b.

Figure 2b: A. Platysma ruptured inferior to mental eminence. B. S10/S3-impregnated pre-cured muscle (1) cut from separate tissue. C. Restorative muscle (1) positioned over rupture site and blended into tissue with silicone adhesive (D, E). F. Additional strips of pre-cured muscle (2) applied along repair margin and blended with silicone (G) to restore anatomical appearance (H-I).

Figure 3: A. Deep prosection of the infratemporal fossa showing a displaced auriculotemporal nerve. B. Auriculotemporal nerve emerges anterior to condylar process. C. Trunk of nerve removed with forceps and moved posterior to condylar process. D-E. Nerve is repositioned with insect needle and reattached to branches with silicone adhesive. F. After silicone has cured, insect needles are removed. G. Auriculotemporal nerve emerges posterior to condylar process.
The auriculotemporal nerve normally emerges from behind the condylar process of the mandible. In this prosection, the auriculotemporal nerve emerged anterior to the condylar process. This was corrected by repositioning the nerve to emerge posterior to the condylar process. The trunk of the nerve had been broken from its two branches, which were left in place just anterior to the auricle. Using an insect needle, the trunk of the auriculotemporal nerve was repositioned behind the condylar neck. In this position, the trunk was reattached to its two branches using silicone adhesive applied with the flat end of a Woodson, and repositioned with forceps and insect needles.

Figure 4a: A. Deep prosection of back region showing infraspinous fossa with detached infraspinatus muscle. B. Detached infraspinatus muscle (1). C. Similar pre-cured skeletal muscle (2) cut to size and shape to replace detached muscle (1). D. New muscle (2) secured to scapula with insect needles, attached with silicone adhesive, and reflected from infraspinous fossa. E. Silicone applied with 24 ml syringe along length of attachment margin. F. Silicone spread along repair margin with paintbrush. G. Strips of pre-cured muscle placed along attachment margin to restore anatomical appearance (H-I).

Figure 4b: A, C. Strips of pre-cured fascia are applied along the attachment margin of the infraspinatus muscle. B, D. Fascia is blended with contours of the muscle.

Figure 4c: A. The attached infraspinatus muscle is cured with S6 Hardener. B, C. S6 Hardener applied to surface and injected into pre-cured muscle with 1 ml syringe. D. Attached muscle is covered with plastic wrap to trap S6 vapor. E, F. Plastic wrap and insect needles are removed.

In this prosection the detached infraspinatus muscle was replaced with restorative skeletal muscle impregnated with S10/S3, of similar color, and cut to similar size. Using large insect pins, the replacement muscle was secured to the scapula, and positioned to reflect from the infraspinous fossa. The muscle was then attached with silicone adhesive applied through a 24 ml syringe along the length of the attachment margin, and spread with a small paintbrush. Small strips of pre-cured, S10/S3-impregnated muscle were additionally cut with a scalpel and inserted perpendicularly along the attachment margin with forceps, to further integrate the new muscle into the infraspinous fossa and restore anatomical appearance.

Small strips of pre-cured, S10/S3-impregnated fascia were applied along the length of the reattachment margin, and contoured to the muscle with a Woodson. This process was repeated as needed to restore anatomical appearances along the margin of repair, and to fully integrate the reattached muscle to the scapula. Using a syringe, 0.5 ml of S6 Hardener was then injected into the muscle and applied onto the surface as a curing agent. The surrounding area was finally covered with plastic wrap to contain the vapor from the S6 solution, and left to cure overnight.

Results

The anatomical appearance of the damaged structures improved remarkably after each of the restoration procedures. The silicone adhesive was compatible with S10 plastinated tissues and self-cured in 12 hrs. In the deep prosection of the head and neck, both the internal and external carotid arteries were easily repaired with silicone adhesive, while reinforced by pins placed inside the lumen (Fig. 1: B-C). Tears on the tunica externa of both arteries were still visible after they were mended with the silicone adhesive (Fig. 1: C). After reattachment of the
arteries, the impregnated fascia applied around the tears blended easily into the tunica externa. This camouflaged all traces of previous damage to the structures. After the silicone adhesive had cured, the fascia provided additional protection and reinforcement to the repaired areas, in addition to helping to recover the original anatomical appearance of the internal and external carotid arteries (Fig. 1: D-F).

The detached platysma from the superficial prosection of the head and neck was positioned to the inferior border of the body of the mandible with insect pins, which facilitated its reattachment with silicone adhesive (Fig. 2a: B). The strips of pre-cured, S10/S3-impregnated fascia blended easily into the muscle and restored the anatomical appearance of the muscle’s insertion into the lower part of the body of the mandible (Fig. 2a: D-E), and beneath its attachment to the mental eminence (Fig. 2b: B-E). Strips of spare, pre-cured muscle were also added along the inferior border of the body of the mandible where the platysma was reattached, and blended into the tissue (Fig. 2b: F-G). This helped to integrate and strengthen reattachment of the platysma to the inferior margin of the mandible.

In the deep prosection of the infratemporal fossa, the auriculotemporal nerve was incorrectly positioned anterior to the condylar process of the mandible (Fig. 3: B). After the trunk of the nerve had been torn away from its two branches, the insect pins allowed the nerve to be repositioned posterior to the condylar process (Fig. 3: C). This repositioning facilitated its reattachment to its two branches with silicone adhesive (Fig. 3: D-F). No further application of uncured fascia was necessary after this step, and the insect pins were removed after the silicone adhesive had cured. The auriculotemporal nerve and its branches were stable, and the usual course of the nerve was clearly demonstrated (Fig. 3: G).

The pre-cured, S10/S3-impregnated muscle selected as a replacement for the detached infraspinatus muscle from the deep prosection of the back was successfully secured onto the scapula with silicone adhesive and insect pins (Fig. 4a: A-F). The subsequent application of pre-cured strips of muscle at the site of reattachment closed any spaces between the replacement tissue and the infraspinous fossa (Fig. 4a: H-I). Pre-cured, S10/S3-impregnated fascia applied along the margin of reattachment blended and contoured easily into the muscle grooves (Fig. 4b). The S6 Hardener reacted with the impregnated S10/S3 silicone-mix and caused the newly-attached muscle to become hardened, dry, and odorless, without leaving behind any surface precipitate. The new muscle was left in a stable and permanent position, reflected from the infraspinous fossa (Fig. 4c: E-F).

**Discussion**

The plastinated prosections used in this study are part of an extensive anatomical teaching collection at NYU Dentistry, and have been used in the classroom since 2005. Periodic repair and restoration to the collection has become incumbent in order to extend the utility and shelf life of the collection over time. The intention of this paper is to illustrate this necessity for other departments using plastinated prosections, and to set a precedent from which to innovate and develop effective restorative methods when these specimens become damaged.

Insect pins were very useful to hold structures in position in order to proceed through the steps of the restoration process. After the repair was complete and the silicone adhesive had cured, smaller pins were easily removed from the tissue with forceps without leaving evidence of their prior usage. Larger insect pins, such as those used to position the infraspinatus in Figure 4a, left a visible hole where it had pierced the muscle prior to the gas-curing step. However, this can be avoided in the future with alternative placement of insect pins, if not by using smaller gauges.

Silicone adhesive is compatible with the S10/S3 impregnation solution used in most plastinated prosections, and therefore can mend broken plastinated structures while maintaining their integrity and relative flexibility. Another benefit of silicone adhesive is that once cured, it can be peeled away from the surface and reapplied in the event that a structure needs to be repaired more than once. Based upon the degree of damage, and the size of broken structures, evidence of previous injury may still be traceable even after being repaired with silicone adhesive. This can be resolved by integrating pre-cured, S10/S3-impregnated fascia to camouflage damage, and restore normal morphology and appearance.

The fascia used may be thin, loose or dense fibrous connective tissue, which is associated with the surfaces of muscles and organs. The pre-cured restorative tissues used in this paper came from the Plastinarium in Guben, Germany, and included fascia, muscle, artery, and nerve. They were shipped to NYU Dentistry upon request for the
exclusive use of restoration and repair of the Department’s anatomical teaching collection.

This tissue is usually cleaned off a plastinated specimen during the “positioning” stage of the S10 plastination process when muscle and neurovascular structures are placed in their desired anatomical position with insect pins before they are gas cured. Rather than being discarded, the fascia can be recycled for the future purpose of rehabilitating damaged plastinated specimens. At this stage, the fascia is still soft, and can be unfurled over damaged tissue and integrated into neurovascular structures and muscles to rehabilitate tears and restore normal anatomical appearances. The “positioning” stage is also an opportunity for further dissection. Superfluous muscles, nerves, and arteries are usually trimmed off the specimen and discarded at this stage. Like fascia, these tissues can also be recycled for restorative purposes, by replacing detached structures. Pre-cured strips of muscle are also easily integrated into host tissue with silicone adhesive, as they are still soft, sticky and pliant.

Alternatively, some departments will restore previously broken structures with an acrylic paint after reattachment with silicone (Raoof, 2014). However, at NYU Dentistry, none of the neurovascular structures in our plastinated collection are painted, though some of the muscles are. Furthermore, the teaching specimens provided by von Hagens Plastination have color-injected vessels and are not painted, except occasionally, muscle. This is an aesthetic choice that we make in our department. We have found that the application of pre-cured fascia is a preferable and aesthetic alternative to acrylic paints for the restoration process. Additionally, pre-cured fascia recovers anatomical appearances of previously broken arteries and muscle, reinvigorates broken structures, and protects the original repair sites.

In all cases, silicone works as an effective adhesive to reattach broken structures, but it does have some limitations. Routine application of silicone adhesive to reattach the arteries in Figure 1 did not conceal evidence of previous damage, as tears in the walls of the arteries were still visible even after they were reconnected. Because the vessels were already injected with a dyed polymer, the insect pins were well supported within the lumen. This provided an effective means of maintaining reattachment between injected arteries after they were reconnected.

Due to the tautness of the platysma in Figure 2b, merely reattaching the broken ends with silicone adhesive could not close the gap produced by the tear beneath the mental eminence. Here, the additional strips of uncured, S10/S3-impregnated muscle were able to blend in with the host muscle and close the gap produced by the tear. This procedure needed to be performed twice to fully close the space.

Only small volumes of S6 Hardener were necessary in order to cure smaller muscles and other structures. Here we used small volumes of S6 (500 ml) within and on the surface of the muscle in a ventilated space. Because the S10/S3-impregnated muscle had been kept at room temperature for 2-3 weeks, the impregnation mixture had already begun the cross-linking process, thus rendering the tissue viscous and pliant before exposure to S6. This slow-cure procedure and exposure to minimal amounts of S6 prevented a precipitate from forming on the surface of the muscle (Weiglein and Henry, 1993). To keep pre-cured, S10/S3-impregnated tissues from cross-linking, these tissues should be stored in a deep freezer at -20°C.

For large structures, volumes of S6 Hardener between 10-100 ml are recommended in order to minimize the potential for atmospheric contamination, and the tissue should be exposed to S6 in vapor form (gas cure). In such cases, a desiccant should be used to absorb excess solution (Weiglein & Henry, 1993).

Pre-cured, S10/S3-impregnated tissues can be made available in those institutions that already dissect cadavers and operate their own plastination laboratory according to the S10 cold temperature technique. It is recommended that spare tissues already impregnated with S10/S3 polymer, but not cured, be kept for future restorative purposes.

The collection of plastinated prosections at NYU Dentistry was purchased for the purpose of education, and with the knowledge that they would be handled by hundreds of students every year. Faculty and students are advised to handle teaching specimens carefully. At NYU Dentistry, we encourage the use of flexible wires by students when exploring and moving aside structures, in order to minimize wear and tear. However, while the technology of plastination itself ensures indefinite preservation, damage to structures and specimens is inevitable. Used periodically, the restoration and repair methods described in this paper can rehabilitate damaged plastinated prosections and allow them to resume their usual anatomical appearance and extend their shelf life and educational value for future use.
References


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TRENDS IN WHOLE BODY DONATION IN A SOUTH AFRICAN INSTITUTION OVER A THREE-DECADE PERIOD (1988-2017): IMPLICATIONS FOR MEDICAL EDUCATION

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Introduction: Dissection and prosection remain the gold standard for the teaching of anatomy to pre-clinical medical students across the world. This has made the practice of whole body donation the cornerstone of medical programs.

Objective: This study aimed at determining the trends in body donation among South Africans, and at predicting the best possible, and most realistic, approach for the teaching of anatomy in the near future.

Materials and Methods: Data from 696 cadavers donated during a three-decade period (1988-2017) were obtained from the files of the Discipline of Clinical Anatomy, University of KwaZulu-Natal South Africa. Data were analysed as percentages, mean ± standard deviation, using Statistical package for social sciences version 24.

Results: Most bodies were donated in the first decade of this study (1988-1997). Funeral services accounted for the major source of donations. Bodies were predominantly in the seventh decade of life (18.8%) and a larger proportion were males (61.6%). The practice of body donation were found to be more among the whites (57.5%) than all the other races. Most deaths according to this study were related to cardiovascular and cardiopulmonary issues (21.6%) and malignancies (11.1%), while a larger percentage were due to “unknown” causes (37.3%).

Conclusions: The study was able to show that the trend in the practice of body donation in South Africa has been erratic, which makes it difficult to predict the number of bodies available for medical education. Alternative approaches to anatomy education such as plastination techniques and computational models need to be sought to ensure sound and uninterrupted medical programs in South African medical schools.
CORROSION CAST OF THE MALE CANINE URETHRA

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Introduction: The male urethra carries urine, semen and seminal secretions to the distal end of the penis. Retrograde ejaculation has been shown to occur in the dog, however, in the author’s knowledge, studies regarding the retrograde flux of the urethral contents to the ductus deferens have not been made. Despite the clinical interest involving the urethra, due to its common pathological conditions, and conventional and minimally invasive surgical protocols, the urethra has been given little attention, in contrast to the abundance of studies on the urinary bladder, ureter and kidney.

Objectives: The purpose of the present study was to create a three-dimensional model of the urethra by means of a corrosion cast, to describe the structure of the pelvic and penile urethra and their relationship to the ducts of the prostate, and to identify if retrograde flux to the ductus deferens can occur.

Materials and Methods: Ten male mixed-breed dog cadavers were used in this study. Dogs were between 1-5 years of age with mean weight of 24 kg (range 18–28 kg). The cadavers were obtained from the Veterinary Hospital of the Autonomous University of the State of México, and were subjected to euthanasia with an overdose of sodium pentobarbital for reasons not related to reproductive or urinary disorders. All animals received humane care in compliance with the Animal Care and Bioethics Committee of the Autonomous University of the State of México. The caudal abdomen was dissected in order to clamp the vesical trigone with Kelly forceps. A 16-gauge vascular catheter was introduced into the external urinary meatus, and 20 ml of yellow epoxy Biodur® E20 was injected, followed by refrigeration at 4°C for 48 hours. The reproductive system was isolated from the body and then corroded using sodium hydroxide (NaOH).

Results: The three-dimensional model obtained was useful to observe the pelvic and penile portions of the urethra. This model can be used to observe the narrowing of the urethra at the level of its ischial curvature, and before the access of the os penis. A large dilation was present at the level of the prostate. Morphological characteristics of the inner surface of the urethra remain imprinted in the cast. These details may be helpful to describe the prostatic ducts draining to the urethra. In three cases, the polymer was able to fill the ductus deferens, which may explain the possibility of retrograde flux of urinary secretions.

Conclusions: The three-dimensional model of the male canine urethra by means of epoxy E20 is a useful tool to perform research on the reproductive tract in the dog, due to its capability to preserve the spaces and contours of it.
SETTING-UP A PLASTINATION LABORATORY IN RESOURCE-LIMITED SETTINGS

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A low-resource setting, or resource-limited setting (RLS), is typically characterized by lack of funds to cover costs for implementation of essential and critical components of projects leading to one or all of the following consequences: limited access to equipment, supplies, devices, capacity, and training, as well as overall retardation in outcomes/outputs. Whether an industry or medical/educational establishment, many institutions in sub-Saharan Africa are poorly funded and thus may fit into this model.

Medical institutions are established to train health professionals for the needs of the country, and therefore this involves infrastructural, technical, and human capacity developments that are tied to adequate funding. The academic activities of an anatomy department thrive with a sustainable repertoire of cadaveric and other materials for training. With dwindling resources, coupled with poor uptake of body donation programs, it becomes difficult to fulfil this mandate. Setting up a plastination laboratory (PL) to generate sufficient and augmented training/research materials becomes imperative. However, it is an expensive venture especially, in RLS.

The design of a plastination laboratory frequently involves technical issues, structural problems of the facility, & especially economic problems that may delay or mar its use, and the possibility of obtaining plastinated anatomical specimens in a brief period of time. We shall be sharing our thoughts and experiences from various African settings with a view towards encouraging other smaller units or labs to start a PL irrespective of the teething challenges in RLS.
THE EVOLUTION IN THE TEACHING OF ANATOMY: PLASTINATION TO THE RESCUE

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Anatomical science and education has progressed so rapidly in the last century, owing to the positive revolution in technological innovations. From the use of paintings, sketches and models, the imparting of anatomical knowledge evolved across institutions, utilizing fresh (unfixed) and then later, formalin-preserved specimens, corrosion casts, plastinated materials, to 3-D computer aids.

These radical transformations have all come with their attendant consequences on the manner of knowledge acquisition, and perhaps the overall goals thereof. Questions are generated, have we reached the crescendo in anatomical innovations with regards to the various modalities adopted in the delivery of anatomical knowledge?

We shall be examining the relative contributions made towards the development of various modalities for the teaching of the subject of anatomy, and perhaps x-ray the role of the plastique technique as an added repertoire.
PLASTINATION APPLIED TO THE CONSERVATION OF CULTURAL HERITAGE: THE ELEPHANT TUSKS OF BAJO DE LA CAMPANA’S PHOENICIAN SHIPWRECK, NATIONAL MUSEUM OF UNDERWATER ARCHEOLOGY, CARTAGENA.

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Introduction: The archaeological excavation of a Phoenician shipwreck at Bajo de la Campana, San Javier, Murcia, Spain, was developed between the years 2007 to 2011 under a collaboration agreement signed between the Institute of Nautical Archeology of Texas A & M University and the Ministry of Culture Of Spain, through the National Museum of Underwater Archeology of Cartagena. Throughout successive campaigns of systematic excavation, the archaeologists documented and raised an extraordinary cargo from the wreck, dated between the 7th-6th centuries B.C. Among the raw materials it carried, there is a magnificent set of 53 elephant tusks and fragments of elephant tusks, some of them with inscriptions. The uniqueness of this archaeological find is that it is one of the few signs of Phoenician navigation in Spain and in the Mediterranean sea, and also it is one of the few examples of ships carrying ivory as a raw material, which is why conservation procedures are not well developed.

Objective: Our main goal was to study the Biodur® S15 technique, at room temperature, on archaeological waterlogged ivory as an alternative to traditional conservation procedures that have been found to be ineffective.

Materials and Methods: Tusk section (SJBC_11_2471_5a), tusk fragment (SJBC_11_2980) and tusk (SJBC_10_1926) were dehydrated in successive acetone baths in a refrigerated chamber at 5° C, to avoid contraction and tension forces. Impregnation of dehydrated tusk specimens was carried out in a vacuum chamber with a mixture of silicone polymer and catalyst: Biodur® S15 and S3, 1%. The pressure in the chamber during impregnation was gradually decreased from 760 mm Hg to 3 mm Hg. This stage was carried out at room temperature, between 18° C and 20° C. After impregnation, specimens were placed in a curing chamber where Biodur® S6 crosslinker was vaporized.

Results: The results obtained have been satisfactory, both in dimensional stability and visual aspect. After treatment, no discernable changes were observed in the physical dimensions and they have acquired the necessary mechanical strength to make study or display possible.

Conclusions: The plastination procedure meets the main objective of water removal and dimensional stability. It is also retractable, a preferred quality in conservation of cultural heritage, but not exclusive, which could be considered as a study option to see the results on ivory. The results after two years after processing enable the authors to validate the Biodur® S15 technique as a conservation procedure in archeology.
GENERAL PRINCIPLES OF DEHYDRATION IN PLASTINATION

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As plastination is defined as the replacement tissue water, both intra- and extracellular, with a curable polymer (i.e. silicone, polyester or epoxy), there is a major problem as water is not miscible with each of these polymers.

In order to make the replacement of water with one of these polymers possible, the water must be replaced with a fluid that is both miscible with water and the specific polymer, and can later be replaced with this polymer. In everyday practice in plastination 2 fluids can be used: ethanol and acetone. They are called intermediate solvents.

During the impregnation stage, this intermediate solvent is replaced by the polymer of choice by means of an increasing vacuum (or a decreasing pressure), making the intermediate solvent evaporate, and being replaced by the polymer. It is preferred that this process, impregnation, proceeds very gradually. As acetone starts to evaporate much earlier than ethanol, acetone is by far the intermediate solvent of choice during plastination. During the presentation we will discuss all the practical pitfalls that can occur during the process of plastination.
PRINCIPLES OF THE POLYESTER TECHNIQUE

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In plastination 2 different kind of specimen are distinguished, 3 dimensional specimens, like normal anatomical specimen, that are normally impregnated with silicone, and thin (<4 mm.) slices, that can be impregnated with either polyester or epoxy. Both polymers have their own characteristics, and field of use. Originally, polyester was developed for its famous distinction between white and gray matter in the CNS, whereas epoxy was used for sections of other organs. Later, many people tried to use epoxy also for brain slices, and polyester for body slices, and with success.

However, there are big differences between these 2 techniques, and because of these differences there are some limitations to both techniques.

Polyester can be used for impregnation right from the factory, no additives need to be used, and hardening or curing takes place by UV-A light. This means that very dark and thick sections will not be penetrated enough by the UV-A light, and consequently will fail to harden. In the end the slices will become very deformed. On the other hand, once impregnated, polyester slices can be kept in the polyester for months (or years), as long as they are kept in the cold. Also, impregnation is not time-dependent, you can start impregnation, stop halfway, and go on after a few days, or even after your holiday. Curing will only succeed well in absence of oxygen, so the specimen can only cure in a completely closed chamber, when in contact with air the curing will fail.

Epoxy has to be mixed to a reactive mixture before impregnation. As soon as the mixing of the mixture is completed, the mixture will start to harden. Therefore there is a limited time window in which the impregnation can be finished, once you start, you must go on. On the other hand, as the curing will go on independent of other circumstances, there is no need to pack the slices in an airtight chamber, just packing them between two foils of polyester will give the slice a nice and smooth surface. In this presentation we will discuss the principles of polyester sheet plastination.
PRELIMINARY ASSAY OF STANDARD S10 PLASTINATION METHOD TO PRESERVE MACROPARASITES SPECIES

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Introduction: Plastination is a well-known process usually employed in human/veterinary anatomy and surgery teaching, but this method has been rarely used for parasites to date. Indeed, only three works have been found in this line, one focused on the human nematode Ascaris lumbricoides, other on eleven species of animal cestoda, nematoda and arthropoda and a previous study by the authors on mature and immature larvae of diptera (Oestrus ovis) using adaptions in the standard methodology.

Objective: The aim of this study was to assay and validate the use of S10 plastination to preserve different macroparasites species.

Materials and Methods: Several specimens of nematodes (Ascaris suum n: 9 and Parascaris equorum n: 5), acanthocephalans (Macracanthorhynchus hirudinaceus n: 5) and trematodes (Fasciola hepatica n: 5) were used during the assays with the standard S10 silicone plastination technique. Half of the specimens of nematodes and acanthocephalans were cut along their cuticle to check if a better exchange during impregnation occurred.

Results: Nematodes presented several morphological alterations related to collapse of their structures: all P. equorum individuals suffered collapse; three of A. suum specimens were successfully plastinated, while the others showed body collapse; three M. hirudinaceus were correctly plastinated, but one parasite presented collapse of their structures, and the other one was broken as a consequence of its fragility. F. hepatica was successfully plastinated (100%), showing similar characteristics to parasites in formaldehyde, alcohol or other traditional preservation method. No relationship was found between results and the use of cuts or not. Further studies are needed to validate damage areas, comparing the abnormal structures of the plastinated and non-plastinated parasites.

Conclusions: Although the plastinated parasites might be quite useful for teaching, it is essential to apply modifications to the standard procedure for each parasitic species, because is not possible to use the same protocol for organisms so different from each other.
Prenatal Development of the Moderator Band with Special Reference to Purkinje Fibers in the Goat (Capra hircus)

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Introduction: Prenatal development of the moderator band was studied in 36 morphologically normal fetuses of the non-descript goat (Capra hircus), divided in 3 groups with equal number (12) in each; Group I (early prenatal < 50 days), Group II (mid prenatal 51-100 days), and Group III (late prenatal 101 days to full term).

Materials and Methods: The heart was fixed in standard fixatives, then washed and processed for paraffin microtomy. Thin (5µ) sections were stained with routine standard fibrocellular stains.

Results: The first histological evidence of juvenile cardiac myocytes and Purkinje cells were noticed in the 4 chambered heart stage at 34 days of gestation. But the moderator band was first observed in the right ventricle of a 42-day embryo. Bundlization and initial differentiation of cardiac muscle fibers and Purkinje cells were observed in 46-49-days embryos. Conduction tissues were ensheathed by the fibro-reticular connective tissue on the 49th day. Grossly identifiable moderator band occurred in the right ventricle on day 51, but in the left ventricle it happened only after 60 days. On day 71 a strong, thick, muscular moderator band grew in the right ventricle. Between 94-99 days the moderator bands of both ventricles assumed typical postnatal-type morphology and disposition. They grew thicker and stronger at a much faster rate in the late prenatal subjects. Cardiac muscle fibers were striated branched and showed intercalated discs. The Purkinje cells located peripherally spread myofibrils and single or multiple nuclei, these mostly occurred in groups surrounded by fibro-reticulo-elastic connective tissue sheath.
AUGMENTED REALITY PRESENTATION OF ANATOMICAL VARIATIONS: EXAMPLE WITH ABERRANT RIGHT SUBCLAVIAN ARTERIES

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Introduction: Clinical recognition of anatomical variations is critical for proper diagnosis and treatment. However, spatial arrangements and underlying mechanisms are difficult to conceptualize. Augmented reality (AR) can provide a novel method to enable rapid and effective understanding of variations.

Objective: The purpose of this study was to develop a computer model of an aberrant right subclavian artery (ARSA) and assess its usefulness within the context of anatomy education.

Materials and Methods: Two ARSA variants were identified during routine anatomy dissections, and quantitative characterization was performed. A plastinated heart was generated and subjected to photogrammetry. Utilizing quantitative features of dissected anatomical specimens, the heart was modeled, polished and viewed within AR space. A video was created utilizing AR projection and a clip of the proposed embryological mechanism of ARSA. Audio descriptions in English and Japanese languages were synced with the video and presented to students (n=161) who subsequently completed a survey.

Results: Students found the clip with AR to be helpful for understanding ARSA (scored as 4.2 ± 0.9; 1, not helpful; 5, very helpful). While four students (2.5%) responded that a textbook/journal article is more useful, 64 (39.8%) believed the clip alone and 93 (57.8%) felt the clip with text was helpful for understanding ARSA. Most (n=154, 95.7%) would use AR tools in the video for learning anatomy, stating benefits that included the ability to visualize and manipulate structures, and good clarity and emphasis on specific structures.

Conclusions: The AR tool and clip, together with traditional anatomy resources are valuable, since important spatial information is provided facilitating a rapid understanding of variations. In the classroom, AR may also be helpful for learning complex normal anatomy concepts. Work is being directed at developing quantitative tools to assess the educational usefulness of AR.
PERSISTENCE OF UMBILICAL ARTERY IN THE MEDIAL UMBILICAL LIGAMENT: CLINICAL ASPECT

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Introduction: Knowledge of anatomic variants on the anterior abdominal wall has often been underestimated. Minimal access surgery (MAS) involves the creation of pneumoperitoneum for improving visualization of the viscera which is established by blind insertion of either a Veress needle or trocar. Since the initial placement of the trocar is the most dangerous aspect of MAS, understanding of the anatomy of this region becomes imperative.

Objectives: To observe the variations in the medial umbilical ligament (MUL) with respect to persistence of the umbilical artery (UA).

Materials and Methods: 24 cadavers were observed during routine dissection (carried out according to the Cunningham’s Manual of Dissection volume II) of the undergraduate students.

Results: The medial umbilical ligaments is the fold of peritoneum raised bilaterally by the obliterated UA, which arise from the anterior division of the internal iliac artery (AlIA). The distal part obliterates to form the MUL, while the proximal part remains patent as the superior vesical artery (SVA). In the present study, 23 cadavers presented with an obliterated UA with normal MUL anatomy, however, in one elderly male cadaver, we observed a small mesentery associated with the right MUL along with a persistent underlying UA. Both the UA and SVA were seen as separate branches of the AIIA. The length of the UA from the origin was 37cm, and it ran inferior to the ureter near its origin, and the vas deferens after it emerged from the deep inguinal ring. A preliminary grading scale for the MUL was proposed by Tokar and Yucel, based on the anatomical appearance of the MUL in 126 patients (aged 28 days - 17 years). These investigators reported 11% of cases with no visible ligament (grade 0), 50% of cases with MUL as a fibrous cord without web formation (grade 1) while 39% of cases presented a fibrous cord with a web in the MUL (grade 2). Here, we propose a new category in the existing classification, with the MUL associated with a mesentery and containing a persistent UA.

Conclusions: A few clinical reports have associated the presence of persistent UA with hydronephros and lower flank pain, highlighting the need for examining the presence of aberrant vessels in the differential diagnosis of distal ureteric obstruction. Furthermore, the possibility of an anomalous UA compressing the vas deferens cannot be ruled out. Besides, awareness of such anatomical variants of the MUL is of relevance as they may expedite a surgeon determining the site of safe trocar insertion in the lower abdomen, preventing technical difficulties during MAS, and also assist in planning appropriate excisions of such structures to provide space for exploration.
THE VALUE OF USING PLASTINATED SPECIMENS FOR TEACHING ANATOMY AT THE UNIVERSITY OF CAPE TOWN

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Introduction: The Department of Human Biology at the University of Cape Town has a fully-equipped plastination laboratory that produces dry, odourless specimens as an alternative to traditional wet specimens for teaching Anatomy. The use of wet specimens is being supplemented with plastinates in consultation with the lecturing staff to ensure that the practical teaching needs are met in courses where whole body dissection does not take place. Plastinated specimens provide several advantages over wet specimens, such as ease of storage, as buckets of harmful formalin are not required. Plastinates are also more pleasant to handle by staff and students as they are dry and gloves are not required.

Objective: The aim was to survey students who are studying Anatomy to find out whether plastinates aid in their learning more efficiently than wet specimens and whether the students support the increased use of plastinates for teaching Anatomy in the practicals.

Materials and Methods: Students studying Anatomy in the first and second year Health and Rehabilitation Sciences courses were selected for the sample. A cross-sectional study was undertaken by means of a five-point Likert scale survey consisting of questions about background information, such as degree registered for, and previous exposure to anatomical specimens. Specific questions regarding the physical appearance of plastinated and wet specimens were included, as well as the students’ opinions about the use of both types of specimens. The survey was anonymous, and ethics approval was obtained from the Human Research Ethics Committee. The scale ranged from 1 (strongly disagree) to 4 (strongly agree) with 0 being “unable to say”. Descriptive statistics were used and the data were represented as percentages, and displayed on bar graphs. The non-parametric Wilcoxon signed-rank test was used to determine whether there were any significant differences in the responses between the first and second year classes.

Results: The response rate was 17%, with a total number of 47 students completing the survey. Previous anatomy exposure was reported in 60% of the sample, namely from high school biology or first year Anatomy courses. There were no statistically significant differences between the responses of the first and second year students for all the questions. Just over half of the students had heard of plastination (51%), with 32% reporting attendance at the Body World’s exhibitions. Most of the students found the odour of plastinates to be less strong than that of wet specimens (83%) and supported the use of them in Anatomy practicals (80%). There was support for the continued use of wet specimens as 51% of students stated that they would not prefer the exclusive use of plastinates for exam revision.

Conclusions: The opinions of the students studying Health and Rehabilitation Sciences support the continued use of both plastinated and wet specimens in the Anatomy practicals in the Department of Human Biology at the University of Cape Town.
TOWARDS A GEOMETRICALLY ACCURATE HUMAN UPPER AIRWAY MODEL: CONTINUOUS POSITIVE PRESSURE DILATION IN CADavers

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Introduction: Obstructive sleep apnoea is a high morbidity condition characterised by repetitive upper airway closure causing intermittent hypoxia. There is currently no intervention that is both acceptable and effective. The full role of different anatomical structures and patterns of deformability is not yet understood.

Objective: Geometrically correct human upper airway models are vital for any flow and deformability studies yet there is no single best method of recreating the human upper airway.

Materials and Method: Appropriate specimen preparation is important in anatomical model recreation. Hollow organs require dilation to increase flexibility and overcome rigor. Continuous positive pressure ventilation (CPPV) has been used in forensics to overcome rigor in the airway prior to forensic imaging, with success. We envisage a role for CPPV in dilation of the human upper airway for a geometrically correct reconstruction. Our project is set within the Department of Anatomy at UKZN. It was granted full ethics BE037/17 from our UKZN Biomedical Research Ethics Committee.

Results: The project is experimental with a control and experimental group involving a total of 10 cadavers purposively sampled. All 10 initially underwent computed tomographic 3D reconstruction of the upper airway, followed by silicone casting for the control group, and CPPV for the experimental group, for 5 days. CT 3D reconstruction for the experimental group was repeated on day 3 and day 5 whilst being ventilated. The experimental group then underwent silicone casting on day 5. Silicone cast and 3D cast analysis was undertaken to measure geometrical correctness, effect of CPPV dilation, and compare silicone casts to 3D casts of same cadaver.

Conclusions: The results set the stage for creation of a geometrically correct HUA model, creating a model suitable for use in further OSA flow and deformability studies, and augment the body of data on HUA reconstruction. The project is on-going.
Epoxy plastination techniques were designed to preserve transparent body sections. Although several techniques have been described, the E12 (Biodur®) is the best known and widespread technique. Some aspects about E12 protocol:

Preparation of specimens and slicing:
Usually we work with fixed specimens to obtain the sections. However, fresh material is also an option. To obtain transparent sections the specimen should be frozen at the lowest possible temperature, at least -70 or -80°C. Thus, 1.5-3 mm thick sections are obtained. The use of -40°C acetone during the cleaning of the sawdust prevents sections from thawing.

Dehydration by freeze substitution and defatting:
The main difference with regular dehydration in plastination techniques is the need to remove the fatty tissue to get the highest transparency, especially in areas of connective tissue. This is done with several baths of acetone or methylene chloride at room temperature.

Forced impregnation:
The impregnation solution employed consists in epoxy E12 plus the hardener E1. The dehydrated sections are immersed in the impregnation mixture, and forced vacuum is performed at room temperature for 6-12 h. Impregnation is completed when pressures reached are below 5 mmHg.

Polymerization:
Polymerization must be done immediately after finishing the impregnation, which prevents the slices from polymerizing in the impregnation chamber. The temperature, combined with the hardener E1 acts as the polymerization agent.

Although this technique has become established as the best choice for learning sectional anatomy as the basis for diagnostic imaging, its main application is in anatomical research. The low refractive index of the epoxy resin E12 with its minimal shrinkage during polymerization makes it the method of choice to study different tissues, in different planes of cutting, from macroscopic to microscopic levels. The absence of manipulation and decalcification means that the topography of anatomical structures are not disturbed. The removal of fat tissue allows the connective tissue, blood vessels and nerves to be identified quite clearly, without any manipulation.
GENERAL PRINCIPLES OF IMPREGNATION (SILICONE TECHNIQUE)

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Concept: This is the most critical step in plastination. The impregnation of a biological specimen with a curable polymer is based on the difference in boiling points of the volatile intermediary solvent and the polymer-mixture. The replacement of the intermediary solvent (acetone) by silicone is done under forced impregnation. Forced impregnation: The physicochemical characteristics of acetone allow, besides acting as a desiccant for dehydration, its progressive substitution by the impregnation solution under vacuum conditions. This solution contains silicone S10 plus a catalyst (S3) that acts as silicone chain extender.

Equipment: The impregnation unit has the follow components: (i) vacuum chamber for plastination, (ii) vacuum pumps, (iii) valves and vacuum tubing, (iv) vacuum measuring devices: Bennert-type manometer vacuum gauge.

Protocol: The acetone within the tissues of the dehydrated pieces is replaced in a controlled way by the impregnation solution. This replacement is done at cold temperature (-15 / -20°C) and under vacuum conditions, therefore it is known as forced impregnation. Adjusting the pressure in the impregnation chamber through the valves makes it possible to control the impregnation speed, by adjusting the manometers to monitor the pressure. The impregnation rate is reflected by the number of acetone bubbles that appear on the surface of the impregnating solution. Fast impregnation results in an imbalance between the amount of acetone removed from the specimens and the volume of impregnation solution that reaches the tissues, and consequently, a higher degree of shrinkage. In general, the impregnation phase is considered complete when the pressure reached is below 5 mmHg, which occurs after several weeks. Small pieces and hollow organs can be impregnated in a week, while solid organs or large items such as whole cadavers may take several months.
A GRAPHIC PIPELINE FOR COMMUNICATING ANATOMICAL VARIATIONS THROUGH ANIMATED ILLUSTRATIONS

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Knowledge of anatomical variations is critical to avoid complications during clinical interventions, as well as to provide a basis for understanding basic morphogenetic mechanisms. Although useful presentations exist in the literature, many comprise photographs or illustrations, often ignoring the complex, spatial three-dimensional relationships. The use of electronic media to present scientific communications represents a major technological advantage for presenting anatomical variations. The purpose of this study is to present a graphics pipeline for generating animations of anatomical variations. The pipeline consists of four steps, including: data collection, 3D-modeling, model polishing, and presentation. Base structures are generated using a hand-held scanner or medical images. The second step, 3D modeling, is achieved using Scan Soft (scanner) or ER3D (medical images) software to render initial models. Polishing is achieved utilizing Maya software (Mudbox) to adjust surfaces, and then converted to Quicktime animations for presentation. Two anatomical variants are presented, including an aberrant right subclavian artery, and a third head of the biceps brachii muscle. Quicktime Movies present the structures with clarity, and can be individually manipulated by the user to examine three-dimensional relationships. For additional realistic presentation, the obj foundation models were ported to a Z-space platform and projected into 3-D space. Individual model elements could be individually manipulated to provide even greater spatial resolution. It is concluded that animations provide a useful approach for visualizing and presenting anatomical variations.
Introduction: Anatomical variations are frequently encountered in the gross anatomy dissection laboratory. Unfortunately, it is unlikely that a program can retain the novel finding for future students to observe. It would be beneficial to retain a model of the anatomical variation for future students to experience. The purpose of this presentation is to present a strategy for retaining novel variations uncovered in a medical gross anatomy course.

Resources: Multiple variations were identified during gross anatomy dissection; these were then plastinated and subjected to digital photogrammetry (agisof.com). Three-dimensional meshes were generated and then polished, based on quantitative measurements recorded from the original dissections using Maya® software (autodesk.com). The model was then ported to Unity-based platforms including Aurasma, Augment, Sketchfab, Hololens (Microsoft.com), Z-Space (zspace.com), and viewed.

Description: The anatomical models were viewed from all perspectives and an understanding of embryological development could be more fully understood. Models could be viewed and manipulated in a collaborative fashion, further promoting small-group learning consistent with the approach commonly pursued by dissection.

Significance: This anatomical variation pipeline represents a novel process for recording and archiving observations from year-to-year within a program. Models could be potentially shared between sites depending on the commonality of Unity platforms.
ETHICAL ISSUES AROUND PLASTINATION OF HUMAN MATERIALS

OGONDA IA

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Human anatomy dissection is a core activity in the Anatomy curriculum offered in our School of Anatomical Sciences, with the majority of the cadavers obtained through the body donation program. The same can be said for most anatomy courses in medical schools on the African continent, with the exception of most cadavers obtained from unclaimed bodies. However, some medical schools do have challenges in securing cadavers for dissection, and in these places there is a need for plastination of the few cadavers received. While the International Federation of Associations of Anatomists (IFAA), through the Federative International Committee for Ethics and Medical Humanities (FICEM), has presented guidelines on acquiring cadavers through the body donor program, this remains a challenge in Africa, due to cultural and religious practices. Most cadaver dissections are premised on Human Tissue Acts of Health Departments in different countries, which are explicit on the acquisition and use of cadavers, but without making reference to plastination. Ethical issues therefore become critical for plastination of human specimens, as it rightly provides an extended period of usage of the cadaveric materials, which is to the advantage of the Anatomy departments. This paper will highlight and discuss questions such as ‘How well informed are the body donors of what their remains will be used for, and how? Are there areas to draw a line? It is envisioned that other ethical questions will be generated for discussion.
RESIN-REPLICA BOVINE AND EQUINE CARPAL AND TARSAL BONES

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Introduction: Osteology is an essential component of the veterinary anatomy curriculum. At the beginning of the 2nd year at the Faculty of Veterinary Science, University of Pretoria, groups of 4 students are supplied with a set of bone boxes which they have to share. The boxes contain all the relevant bones of the equine, bovine, canine and ovine species. For convenience, the carpal and tarsal bones of the equine and bovine species are arranged in their normal anatomical position, and attached to each other by means of string. These bones are of specific importance for the veterinary surgeon. The current teaching situation creates a problem, as 4 students have to share these bones during the year and then return them at the end of the course. This problem is exacerbated by a dramatic increase in student numbers from 140 to 220. The ideal solution would be to provide each student with a set of carpal and tarsal bones which they could keep permanently. Since it was not a viable option to obtain large quantities of animal bones, it was decided to make replicas using the resin-replica technique.

Materials and Methods: An adult horse and ox were donated to the department, and the carpal and tarsal joints removed. The soft tissue was manually cleaned from the bones, after which they were boiled in water for 3 days. The bones were then cleaned thoroughly and placed in a peroxide solution for 3 days, after which they were taken out and left to dry. They were then defatted by boiling in trichloroethylene for 3 days. Each individual bone was then placed in a container into which silicone was poured. The silicone was allowed to cure, after which the bone was removed leaving an exact negative mould of the bone. Resin was then poured into the mould and allowed to cure. The dry resin-replica was removed and refined using a sharp blade until it resembled the original bone. The resin bones were arranged in their normal anatomical position and attached loosely to each other by means of string threaded through holes drilled in the bones.

Conclusions: The silicone moulds, if made correctly, can be used to produce large numbers of resin replica bones of high quality without sacrificing animals.
DIFFERENT DEPLASTINING METHODOLOGIES FOR MAKING HISTOLOGICAL SECTIONS OF SPECIMENS PRE-PLASTINATED WITH BIODUR® S10 / S3

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Introduction: Plastinated specimens have advantages over organs preserved in formalin including: low toxicity, odorless, dry, clean and durable specimens. One of the most interesting and potentially useful qualities of silicone-plastinated tissues is the preservation of structures at the histological level. This implies that the specimen can be preserved in a form that is easily stored, while maintaining the full potential for future histological and histopathological studies.

Objective: The objective of this study was the development of protocols for the preparation of histological slides from plastinated specimens with BIODUR® S10 / S3 technique.

Materials and Methods: For this study, samples of aorta, heart, and kidney of pigs were used. Four treatments were made to investigate protocols for light microscopy (LM). For comparative use, non-plastinated tissue was used (treatment 1), as follows: immersion in 10% formalin for 48 hours at room temperature. After fixation, tissue samples were processed for LM histology. For treatments 2, 3 and 4, samples from specimens plastinated with Biodur® S10/S3 were used. Treatment 2: plastinated fragments were directly embedded in paraffin without previous deplastination and blocks were made. Treatment 3: plastinated tissue samples were deplastinated by immersion in 99% ethyl alcohol for 24 hours, and then in methylbenzene for 48 hours. After deplastination, the fragments were processed for LM histology. Treatment 4: plastinated samples were deplastinated in xylene for 36 hours and processed for LM histology. Serial sections of 5 µm thickness were obtained in the microtome for each specimen, and stained with hematoxylin and eosin or Verhoeff techniques.

Results: Histological results from the different treatments were compared. Plastinated (treatment 2) and deplastinated with methylbenzene, and desplastinated with xylene (treatments 3 and 4) sections revealed a similar preservation of the histological characteristics of the tissues as fixed tissue (treatment 1), but with low affinity to hematoxylin. In the kidney, the integrity of the renal capsule was preserved in all treatments. The renal cortex showed damage, and the epithelium of the renal tubule showed some retraction in treatments 3 and 4. In the heart, changes in the structure of the myocardium were visible in treatments 2, 3 and 4. With the aorta, it was not possible to visualize the vasa vasorum in the adventitial tunica following treatments 2, 3 and 4, and there were areas of refraction in the connective tissue. All treatments showed elastic lamellae relatively organized with Verhoeff stain.

Conclusions: We observed that deplastination with xylene and methylbenzene both produced a material similar in quality, and similar to plastinated tissue.
EXPRESSION OF cKIT PROTEIN AS A PROGNOSTIC MARKER IN GASTRONEUROENDOCRINE TUMORS

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Introduction: Gastroneuroendocrine tumors (GNET) are rare, with an incidence of 10-30% of all carcinoid tumors. The molecular mechanism and progression of the disease remains unclear. These tumors are malignant endocrine neoplasms that present diverse clinical behaviors. Therefore, identification of biomarkers of GNETs is important for stratification of the prognosis of patients. Recently it was reported that cKIT (a transmembrane receptor tyrosine kinase) has an important prognostic role in various solid cancers and pancreatic NETs. So, we aimed to evaluate the expression of cKIT in GNETs.

Objective: To study the prognostic significance of cKIT in gastroneuroendocrine tumors, and its correlation with histopathological findings.

Materials and Methods: Five surgically resected NETs were obtained after taking ethical clearance from the GI surgery department, AIIMS, New Delhi. Clinical history and pathological findings were recorded. Tissues were fixed in 4% paraformaldehyde, and paraffin blocks were processed for immunohistochemistry. Rabbit monoclonal antibody (1:200) was used to assess the expression of cKIT using the streptavidin biotin complex method. Images were captured using Nikon Ti-S microscope, and intensity was analysed.

Results: Each NET was graded according to WHO guidelines, and the Ki67 index was calculated. Expression of cKIT was observed in the cytoplasm. The intensity of cKIT expression was low in grade1 tumors whereas it was high in grade 3 tumors indicating the variable expression of cKIT.

Conclusions: Expression of cKIT correlates very well with WHO grading and Ki67 index. Therefore, cKIT may be used as prognostic marker, and possible molecular target, for therapy in patients with NETs.

THE MORPHOLOGY OF THE MAXILLARY AIR SINUS UTILISING 3D RECONSTRUCTED MODELS

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Introduction: The maxillary air sinus varies according to age, however, there are limited studies that have illustrated its 3D form over time.

Purpose: This study aimed to classify the maxillary air sinus by the shape, number of septa and scallops in a 1 to 25 year age group, utilising computerized tomography (CT) scans and 3D reconstruction.

Materials and Methods: CT scans (n=480) were reviewed from the picture archiving and communication system (PACS) of the state and private hospitals in Pietermaritzburg and Durban KwaZulu-Natal (KZN), South Africa. The sample consisted of 276 males and 204 females, 1-25 years of age, and of two population groups, black African and white. Morphological traits such as the presence of the air sinus, scalloping, and septa within the air sinuses were categorised. In addition, the shape of the 3D model of the air sinus was analysed anteriorly (coronal) and laterally (sagittal) adapting the classifications by Kim (1962) and Kim et al. (2002).

Results: The maxillary air sinus was present bilaterally in n=477 individuals (99.4%). Five different anterior shapes viz. Type 1 (triangular), Type 2 (inverted triangle), Type 3 (square), Type 4 (irregular) and Type 5 (rectangular) were identified in the anterior view. The shape was associated with age and population groups (p<0.05). In the lateral view, the maxillary air sinus appeared to be quadrilateral with differences noted along the inferior wall. Intrasinus maxillary septa were more evident in the anterior region of the maxillary air sinus (27.9% right; 28.5% left). The maxillary septa were more common in females (37.9% right; 39.4% left) than in males (28.5% right; 30.3% left). They were also more commonly observed in the white cohort (63.8% right; 68.1% left) than in the black African cohort (29.1% right; 30.5% left). Scalloping in the axial plane from above along its anterior border was also observed.

Conclusions: An in-depth classification of the morphology of the 3D form of the maxillary air sinus according to age (1 to 25 years) was established. The shape of the air sinus changed in the form according to age. Laterally, the shape was related to the development of the teeth, as the inferior wall of the air sinus was classified. Surgically, the air sinus morphology is essential for dental procedures such as sinus augmentation or dental implants, and anthropologically, in forensic identification.
VITAMIN K-DEFICIENCY AFFECTS SPERMATOGENESIS IN SPRAGUE-DAWLEY RATS

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Introduction: Vitamin K deficiency in extrahepatic tissues such as the bone and the heart contribute to conditions such as osteoporosis and cardiovascular disease respectively. However, its impact on some other extrahepatic tissues, like the reproductive system remains largely unknown.

Objective: This study investigated the impact of dietary vitamin K deficiency on the testes of Sprague-Dawley rats.

Materials and Methods: Histological examination was carried out on testes fixed in modified Davidson fluid. Semen samples obtained from the cauda epididymis were used to analyse the semen of the Sprague-Dawley rat. The experiment was in three phases (two, four and eight weeks). In each phase, there were two groups (control group and warfarin-induced vitamin K-deficient group (VK def)) of five rats each. All the rats were allowed to acclimatize for two weeks before the commencement of the study. Quantitative data were analysed using one-way ANOVA to compare variables, and results presented in tables and figures.

Results: The results show histopathological changes in the VK def group ranging from delayed spermiation, the presence of multinucleated giant germ cells in the tubules, exfoliation/degeneration of germ cells, poor spermatogenic activities, low sperm count, poor motility, and increased abnormal sperm morphology, when compared to the control groups.

Conclusions: The findings of this study clearly demonstrated that dietary conditions (e.g. vitamin K deficiency) in the body negatively impact on the male reproductive capacity.
GENERAL ISSUES OF SAFETY IN PLASTINATION

SCHILL V

BIODUR® Products GmbH, Im Bosseldorn 17, Heidelberg, Germany

When people intend to start plastination in their institution they are sometimes not aware of the scope of equipment, auxiliaries and chemicals they need. And, to an even lesser extent, are they aware of the potential hazards which arise from the plastination activity. Special chemicals may possess acute or chronic health hazards. Acetone, which is used for dehydration and defatting, is a flammable liquid and therefore brings about fire and explosion hazards.

In this presentation, information is given about the characteristics of the most commonly-used chemicals in plastination. Technical room ventilation or workplace ventilation is required to keep the concentration of hazardous vapours below their respective concentration limits. Personal protective equipment must be used to allow for safe work when handling these substances.

Avoiding the risk of fire and explosion caused by handling of acetone or other flammable solvents is achieved by a bundle of measures: proper laboratory furnishings (ventilation system, electrical installations, etc.) are of importance as well as the equipment used for plastination. Furthermore, the scale of your work determines the safety measures: running a large-scale plastination lab, where you may store and handle several cubic metres of acetone, understandably requires other devices and utilities than working at a smaller scale, with a small equipment package where you dehydrate your specimens in barrels of just 20 or 30 litres of acetone.
SILICONE TECHNIQUE AT ROOM TEMPERATURE

STARCHIK D

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Purpose: There are two silicone plastination techniques all over the world. The classical one (S10) was introduced by Gunter von Hagens in 1977. It uses a reactive silicone impregnation mixture, and needs a freezer to keep it cold for slowing down polymerization. The second technique was proposed by Daniel Corcoran & Dow Corning Corporation in 1998, and it uses an unreactive silicone mixture, and carries out impregnation at room temperature (RT). Cold and RT silicone plastination techniques have differences in polymer components in the impregnation mixture, and also in the sequence of their combination in the curing stage. Because the RT technique is less popular than the cold method, it is advisable to know how to realize RT plastination stages, and what features those plastinated specimens have.

Materials and Methods: We plastinated a variety of organs, regions and whole body specimens with the RT technique using standard procedures (dissection, dehydration, defatting, impregnation, curing), and evaluated the advantages and shortcomings of this method. Criteria used for evaluation included shrinkage, duration of impregnation and curing, quality of plastinated specimens, the need for extra equipment and its maintenance, as well as other cost considerations. Cylindrical core samples of parenchymatous organs were used to efficiently evaluate shrinkage and plastination duration. Core cylinder volume was evaluated at the end of each stage of the process by fluid displacement.

Results: The first three steps for the RT procedure are identical to the cold technique, and the difference is in the impregnation and curing steps only. Room temperature impregnation composition consists of 93% silicone and 5% cross-linker. That is not a reaction mixture, and there is no need to keep it in a freezer. The molecular weight is about 500, and 12-second dynamic viscosity. The average shrinkage calculated for tissue cores plastinated by the RT technique (16.2 ± 1.49 %) was 1.5 times less than by the cold method (p < 0.05). The total duration of impregnation and curing stages of samples for the RT plastination was proved to be 1.54 times shorter than that of S10 technique. The silicone impregnation-mix for the RT technique, because of its low viscosity, drains very easily from impregnated hair, fur, and feather specimens, which is a large time saver. Hollow organs and body part specimens plastinated by the RT procedure were less flexible, more fragile, and harder after curing than those made with the cold technique. There is no need for an additional freezer for the impregnation vacuum chamber, or a special chamber equipped with a fan, an aquarium pump and desiccant for RT technique.

Conclusion: The specimens plastinated with the RT technique are less flexible and elastic, but this process allows production of good quality specimens with minimal shrinkage, and in a shorter period of time. This method is preferable for whole brain, parenchymatous organs, body parts, fetus, fur/hair/feather-covered specimens, reptiles & fish, as well as for long-time formalin-fixed specimens, for archaeological and fossil objects. The room temperature plastination laboratory is more economical to set up.
Purpose: Plastination was invented and developed as a technique for preserving biological tissue, and producing anatomical specimens for studying human and animal anatomy. The added potential of plastination for clinical research was discovered later. Clinical plastination is a special area of scientific exploration and clinical education, with particular interest in the field of applied medicine.

Materials and Methods: Different organs (hearts, lungs, brains etc.) and body parts were taken by autopsy from cadavers, and fixed in formalin. We used the room temperature silicone plastination, and epoxy technique standard protocols for producing demonstration specimens for particular clinical requirement, and for anatomico-clinical research. Injection of colored silicone reactive mixtures was used to contrast arteries and veins. Prosthetic heart valves, rings, electrodes, and stents were implanted during dissection or after curing. We used cutting of hard epoxy-impregnated anatomical blocks to get thin sheet plastinated slices of organs with metal and plastic implants and devices.

Results: Silicone plastination has some innovative approaches to enhance the quality of educational process of clinical disciplines. Three modifications of silicone plastinated specimens were developed: 1) pathological organs to demonstrate diseases, congenital and acquired defects; 2) modified types of surgical dissections for studying clinical anatomy; 3) organs and body parts with implanted metal or plastic construction, valves, prosthetic devices and electrodes. Silicone plastinated specimens are useful for diaphanoscopy, endoscopy of joints, gastrointestinal tract and branches, radiography, CT and MRI tomography. Sheet plastination techniques with epoxy resin have more advantages for clinical research than silicone plastination. It may be used for studying topographical anatomy and tomography. Despite it being more complicated than the silicone plastination technique, the modified epoxy method is the method of choice to research stents in coronary arteries and metal elements in bones.

Conclusion: Clinical plastination brings new facts in clinical research, and provides new opportunities for using plastinated specimens to expand the clinical manner of thinking. It could be made available in clinical centers to allow improved effectiveness of teaching of ultrasound, and radiographic and surgical anatomy and techniques. It is advantageous to combine routinely-used diagnostic and surgical procedures with plastinated specimens, as it gives a better understanding even to the specialists in terms of clinical necessities. Sectioning hard anatomical blocks plastinated with the epoxy technique offers great new opportunities for anatomical and clinical research.
MULTI-DISCIPLINARY USE OF A HUMAN PLASTINATION MODEL FOR GROSS ANATOMY REVIEW

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Introduction: Plastination is a unique process that provides a direct representation of the human body. Human models are advantageous for improving comprehension of gross anatomy concepts because real time interpretation and familiarization of structures can easily be obtained, compared to the use of traditional models. Traditional models are limited because they only provide a 2D view. The ability to use the same human model in various levels of education, such as high school, undergraduate, and more specifically, graduate, physician assistant, and medical school curriculum, is what makes them distinctive and convenient.

Objective: To create a human plastinate that displays anatomical relationships and high-yield learning concepts related to human gross anatomy as an effective instructional tool, specifically focused towards medical students.

Materials and Methods: Wet dissection was performed using a donor obtained through the Lincoln Memorial University – DeBusk College of Osteopathic Medicine’s Anatomical Donation program. The view of the specimen extended from the first cervical vertebra to the mid-humeri and mid-femora, including focal dissections of the thorax, abdomen, and pelvis. The posterior aspect of the donor integrated superficial and deep muscular dissections of the back and gluteal region. The dissection was thoughtfully rendered to reveal anatomical relationships that are spatially and visually relevant for learning medical gross anatomy. The dissection also integrated concepts that can be difficult for students to assess in standardized wet dissections, as well as concepts thought to be high-yield in patient care.

Results: The following items can be appreciated with our model (and are not limited to): the structure and relationship of the first and second cervical vertebrae, the path of the vertebral artery through the cervical vertebrae, the exit of the brachial plexus between the scalene muscles, the muscles of the anterior neck, the spatial arrangement of the heart and lungs in situ, the intercostal nerves and arteries of the anterior chest, the structures of the anterior chest wall, the chambers of the heart, the pleural spaces and their relationship to the diaphragm in the thoracic cavity, the major branches of the abdominal aorta, the kidneys, the paracolic gutters in the posterior abdominal wall, the superficial and deep muscles of the back, the superficial and deep muscles of the gluteal region, the structures of the femoral triangle, and various muscles of the proximal upper and lower limbs.

Conclusions: This human plastinate can easily be presented to coincide with variable curriculum objectives. While this model can be utilized to build the foundational groundwork of human gross anatomy in high school and undergraduate studies, the major aim is to provide professional students (graduate, physician assistant, and medical) with a model that will advance their more intricate understanding of the human body. Students of medicine will be able to apply clinical scenarios to various anatomical structures and reinforce highly important material related to direct patient care.
ANATOMICAL AND DIAGNOSTIC IMAGING STUDY OF THE MEDIAL ASPECT OF THE CANINE ELBOW JOINT

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Introduction: Anatomic, ultrasonographic (US) computed tomographic (CT) and magnetic resonance imaging (MRI) studies of the canine elbow joint have been reported separately.

Objective: The purpose of this study was to correlate the images resulting from the evaluation of the canine elbow joint by means of high frequency ultrasonography, CT, MRI, and plastinated anatomical sections, obtained on the same planes used in the imaging protocols.

Materials and Methods: Anatomical study: 10 forelimbs obtained from 5 adult German Shepherd-crossed breed dog (GSD) cadavers were frozen at -70°C to obtain transparent sections (2mm thick) on the same planes as the imaging studies; anatomical sections were preserved using the E12 plastination technique. Ultrasonographic study: 10 elbow joints from 5 adult GSD dogs were evaluated using an 18 MHz linear array transducer. For the CT study: 6 elbow joints from 3 adult GSD dogs were evaluated, and reformatted images were obtained on the same planes as the ultrasonographic study. Magnetic resonance study: 6 elbows joints from 3 adult GSD dogs were scanned. Correlations between imaging techniques results and anatomical sections were assessed.

Results: The correlation of anatomical sections and imaging techniques allowed an accurate identification of multiple soft tissue and bone structures. High frequency US accurately identified the insertion tendons of brachialis and biceps brachii muscles, the medial collateral ligament and the medial coronoid process, whilst CT recognized the cortical and subchondral bone of the MCP, the trochlear notch of the ulna, the radial incisures, the anconeal process and the humeral condyles. The MRI assessed soft tissue structures such as cartilage, the flexor muscles and their tendons of origin, the course of the medial collateral ligament (MCL), and the insertion tendons of brachialis and biceps brachii muscles. There was an excellent correlation between the images from diagnostic imaging techniques and the transparent anatomical sections.

Conclusions: Our results support those of previous publications. This work, however, combines anatomical plastination and three diagnostic imaging techniques at once. The use of a high frequency transducer provided a greater ultrasonographic resolution of soft tissue structures allowing a better assessment and identification of tendinous structures. By means of MRI the course of the MCL extending to the fibrous sheath of the biceps complex was described for the first time. Correlation between plastinated anatomical sections and images from three different diagnostic imaging techniques leads to a more comprehensive understanding of the canine elbow joint. Moreover, it demonstrates the high value of the plastination techniques as a tool, not only for education, but for a better understanding of the clinical anatomy of the canine elbow joint.
The 19th International Conference of Plastination
July 18-22, 2018 - Dalian, China

This year’s conference is hosted by Dalian Medical University and is designed to provide an innovative and comprehensive overview in sheet plastination and silicone techniques, with the objective of allowing global attendees to mix and exchange knowledge and ideas. During the conference you will also be invited to: the Mystery of Life Museum, the unique museum exhibiting thousands of plastinated animals and human bodies; and Dalian Hoffen, the world-wide largest plastination lab.

From July 15th to 18th, 2018, the 3rd Dalian International Workshop on Plastination will be held prior to the major conference. This preliminary event will offer you an opportunity to touch the authentic plastination technology and exchange your ideas with experienced technicians and experts.

The ICP2018 Dalian is now calling for papers. Appropriate abstracts are limited to applications and innovations on plastination techniques. Abstracts focusing on clinical anatomy, anatomical research, anatomy education, and plastination-based veterinary anatomy are highly welcomed. You will find detailed information on the homepage: www.icp2018dalian.org

We look forward to your active support and participation at the ICP 2018 in Dalian, come and join us, see and be seen, and advance the technique of plastination. Visit www.icp2018dalian.org and register yourself at the early bird price!

More questions you can contact us by sending email: ICP2018@dmu.edu.cn

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Journal of Plastination
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(Revised July 2017)

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Title Page
Abstract with keywords
Text
• Introduction
• Materials and methods
• Results
• Discussion
References
Figure Legends

Title Page
The first page of the manuscript should include:
• Title of paper
• Each author’s name
• Institution from which paper emanated, with city, state, and postal code. Each affiliation should be listed as a separate entity, with a superscript number that links it to the individual author.

For example:
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• Corresponding Author’s name, address, telephone and telefax numbers, and e-mail address.

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It is the corresponding author’s responsibility to notify the Editorial Office of changes of address. Only the corresponding author should communicate with the Editorial office for matters regarding each manuscript.

Abstract & Key Words
The abstract should be no longer than 250 words. It should contain a description of the objectives, materials and methods, results, and conclusions. The abstract should include a section on technique/technical development if the paper is significantly technical in nature. The abstract must be written in complete sentences and be intelligible without reference to the rest of the paper. No references should be used in the abstract.

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Text
The body of the text should be written using American English spelling.

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References
- References to published works, abstracts and books must include all that are relevant and necessary to the manuscript.
- Citations in the text should be in parentheses and listed chronologically; e.g. (Bickley et al., 1981; von Hagens, 1985; Henry and Haynes, 1989) except when the authors name is part of a sentence; e.g. "...von Hagens (1985) reported that..." When references are made to more than one paper by the same author published in the same year, designate each citation as 1999 a, b, c, etc.
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- Legends for all figures should be brief, specific and not be a substitute listing for the result section, and appear on a separate page at the end of the manuscript, following the list of references.
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- All tables must be cited in the text and have titles. Table titles should be complete but brief. Information other than that defining the data should be presented as footnotes.
- Create tables using the table creating and editing feature of Microsoft Word. Do not use Excel or comparable spreadsheet programs.
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The corresponding author should include a statement disclosing any financial or other substantive conflicts of interest that may be construed to influence the results or interpretation of the manuscript. All sources of financial support for the project should be disclosed. Where there are no conflicts of interest, a statement to that effect should be included.

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References
