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### 3-D VISUALISATION OF THE RETROBULBAR ORBITAL SEPTA USING BIODUR E12<sup>®</sup> AND BIOVIS-3D SOFTWARE

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**Introduction:** The retrobulbar fat body in the orbit is supported by a network of collagenous septa that support the ocular adnexa, and aid in coordinating precise eye movements. The exact anatomical nature of these septa and their contribution in ocular motility are not completely understood, but they are thought to act as pulleys for the extra-ocular muscles. This has implications for orbital pathology and surgery.

**Objectives:** The aim of this study was to create 3D models of the retrobulbar fat septa in the human orbit, using epoxy resin and 3D serial reconstruction software, in order to compare the morphology of the septa in left and right orbits, and between individuals.

**Materials and Methods:** Four formalin-fixed human orbits were dissected, decalcified, dehydrated in acetone at -20° C, and impregnated in Biodur<sup>®</sup> E12 epoxy resin. Serial sections (0.3 mm) were cut with a slow-speed diamond saw, stained with Gomori's trichrome for elastin and collagen, and photographed with a digital SLR camera. BioVis3D software was then used to create 3D models of the fat septa.

**Results:** This project generated four x 3D reconstructions of the connective-tissue fat septa, which could be rotated on all axes. The individual reconstructed structures could be isolated and manipulated. Using 3D reconstructions and serial histological sections, common characteristics and variations of connective tissue septa among different orbits were described.

**Conclusions:** Orbits from the same individual were noted to share a similar arrangement and areas of condensation of fat septa. Although sharing broadly similar morphology, the orbital septa of different individuals displayed variations in thickness, density and fine arrangement. The results reported here could serve as the groundwork for defining the normal anatomy of the septa, and for investigations into the clinical and surgical implications of the variations between individuals.

This study received no outside funding.

## ESTABLISHING A PLASTINATION LABORATORY MAY REDUCE RUNNING COSTS FOR THE VETERINARY ANATOMY SECTION AT CVAS, JHANG, PAKISTAN

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**Introduction:** Plastination is a laboratory preservation technique applied to specimens so that they can be used as models in the study of anatomy, for both undergraduate education and research purpose. There are several limitations, including financial constraints, in replacing traditional veterinary anatomy preservation and dissection with the plastination technique in developing countries, because establishing such high-tech laboratories, equipped with costly infrastructure, needs a lot of capital investment.

**Materials and Methods:** Animals can be purchased from the local market for preserving through the standard silicone plastination technique, by following von Hagens' previously described method.

**Results:** This technique may reduce the exposure to various harmful gases and toxic fumes, and provides fixed, non-perishable, long-lasting veterinary anatomy specimens. After plastination, the preserved organs and animal bodies can be used for undergraduate veterinary anatomy teaching for several years at the College of Veterinary and Animal Sciences (CVAS), Jhang, Pakistan.

**Conclusions:** Hence, provision of equipment for plastination in the Anatomy Lab, CVAS, Jhang, will act as a double-edged sword, because, on the one hand, it will protect the environment and health of veterinary anatomy professionals as well as veterinary students, and on other hand, it will reduce the running costs and budget for the purchase of healthy and expensive animals and toxic chemicals essentially needed for the dissection and embalming of equine, bovine, canine, and avian species on a yearly basis.

**Grant support:** This work will be supported by Institutional Strengthening and Upgrade of Labs and Libraries number HEC/ACAD/ISULL/2017/1323; the application is under processing.

**PRE-IMPREGNATION RATE OF FAT REMOVAL BY ACETONE DURING PLASTINATION**

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**Introduction:** It is well known in the plastination community that the dehydrating and defatting phases are not determined by quantitative measurements. Samples are soaked in acetone for varying intervals of time while using the scientist’s discretion to determine when and how often the acetone bath should be changed. This makes the process susceptible to human error and an imperfectly dehydrated/defatted sample. To minimize this inevitable error, measurements were taken to ascertain the rate of penetration for acetone on a given sample, which can be corroborated by histological images. Additionally, the overall effectiveness of the acetone was measured by collecting the total amount of fat released after a set time interval.

**Materials and Methods:** Four x 7.6 cm (three inch) cubes of fat were collected from the lower back of one cadaver, and their weight recorded. The lower back was chosen because of its low concentration of vessels and nerves. Samples were soaked in cold (-25° C) acetone, and then room temperature (25° C) acetone for 21 days each. Acetone was changed at 7-day intervals, and the “dirty”/used acetone was collected and total fat content was determined using a rotary evaporator, and recorded. After three cold changes of acetone, the samples were deemed to be dehydrated (>99% purity of acetone, the “dehydration phase”). The samples were removed from the cold temperature into room temperature for another 21 day soak (a “defatting phase”) with three x 7-day interval changes. The dirty acetone was saved, and fat content determined using a rotary evaporator, and recorded. Total fat extraction was measured from the acetone/fat-mix remaining in the evaporator (approximately 50 ml). This mix was placed in a fume hood to allow complete evaporation of the acetone from the fat. Twenty-four hours later, the remaining fat was weighed and total fat extraction was recorded.

**Results:** The initial weight of each sample was as follows: Sample 1: 107.14 g; Sample 2: 74.19 g; Sample 3: 105.47 g; Sample 4: 83.68 g. Figure 1 illustrates the results of 42 days of acetone soaking. In order to compare the samples, the weight of fat extracted was divided by the initial weight of the sample to give a percentage (range 0.38% to 38.46%, dependent on time). The rate of change between each time interval was calculated by using the “% of fat extracted” as data points. These K-values were then used to compile a line of best fit representing all four samples’ fat extraction rate over time (Table 1).

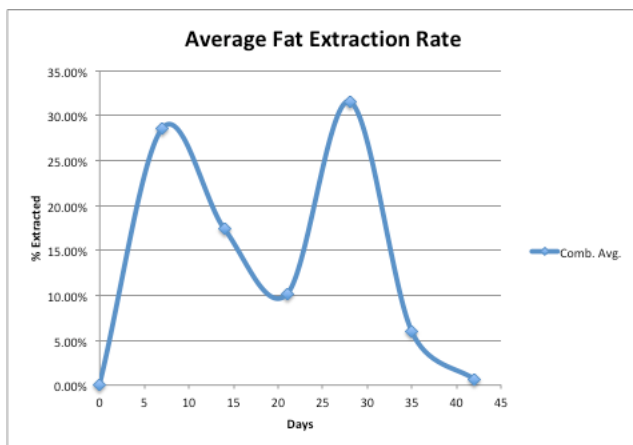


Figure 1

K-values	
Days	Slope
0-7	4.0789
7-14	-1.6014
14-21	-1.0296
21-28	3.0579
28-35	-3.6575
35-42	-0.7586

Table 1

**Discussion/Conclusion:** Based on these data, several points can be concluded. Dehydration was complete after three weeks and samples' density was over 99%. This indicates that the samples are dehydrated, and defatting should be started. This 21-day period is interesting because when you compare it to the defatting phase it disproves the initial assumption that the defatting phase constitutes the majority of the fat loss. In other words, the dehydrating phase sees more defatting than the actual defatting phase. This is supported by the fact that it takes seven less days to reach <10% fat extracted. Additionally, it is concluded that the presence of density readings should be accompanied with each step to ensure that dehydration and defatting is fully complete. Finally, we find that the plastination community should adjust their procedures according to these findings in order to optimize aesthetic results and effectively save time. The degree to which dehydration and defatting should be lengthened and shortened respectively, however, is still elusive due to our samples not being representative of *every* potential plastinated specimen. Furthermore, we can safely say that our goal for quantifying a "successful extraction of fat" was achieved and supported by density readings with the change in slope of fat extraction. It is true that fat extraction can be proven quantitatively.

By collecting measurements of fat extraction during various times in the plastination process, this study provides evidence of acetone's efficiency in penetration of tissue samples. This offers incredible value to the plastination community, because of the ability to point out where time and money can be saved, while also guaranteeing optimal aesthetic quality of the tissues. This project also has the opportunity for extensive follow-up studies. Different dehydrating/defatting agents and procedural techniques can be compared to the ones demonstrated in our study to determine which is more effective. Samples can be taken from different locations on the body and from various organ systems to show how different tissue compositions affect fat extraction. Studying changes in temperature during different time intervals could also reveal further information on fat extraction. Finally, histological imaging should be incorporated into this type of study. By coupling imaging with our study's extraction data, we can observe cellular changes as the fat concentration within the cell changes. There are a plethora of possible combinations to be explored, and what we learn gives us the opportunity to perfect the plastination process and expand the plastination society's knowledge as a whole.

## SILICONE-INJECTED CADAVERIC HEADS FOR NEUROSURGICAL DISSECTION

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**Introduction:** Surgical simulation using cadaveric human heads is one of the most valid strategies, and is still considered to be the gold standard for *ex-vivo* simulation among all the models available for neurosurgical training. The educational value of these cadaveric heads for neurosurgical dissections can be enhanced by the injection of colored dyes in the intracranial vascular tree. The knowledge of vascular anatomy, being integral to all the neurosurgical procedures, gets more clearly defined in these injected specimens. The technique of silicone injection in the cerebral vasculature of human heads has been reported in the literature. However, the technique and materials for the injection of blood vessels of cadaveric human heads in Indian conditions have not been standardized.

**Materials and Methods:** Four freshly-donated human cadavers were used. The technique of dye injection was standardized in goat heads, and subsequently translated into humans. In the human cadavers, the carotid arteries and internal jugular veins were dissected out from the carotid sheath, whereas the exposure of vertebral arteries required deeper dissection of the neck. The arteries were then irrigated with warm normal saline till the returning fluid was clear and free of blood. This was followed by perfusion with the embalming solution. The vessels were then clamped for 30 minutes to one hour. The silicone (red and blue) dyes were mixed with the catalyst, polymerization time was standardized for the individual dyes and then injected into the common carotid arteries, vertebral arteries and the internal jugular veins bilaterally. The injection was stopped once it was ensured that the entire vascular tree was filled with the respective dyes. The cadavers were then decapitated after one week, and then immersed in fixation solution consisting of 10% formalin and 20% ethanol, for 30 days.

**Results:** A small craniotomy was performed around the vertex of the cranium. The condition of the brain and quality of the injection in the meningeal and cerebral vessels was observed. All the injected cerebral vessels, including external and internal carotid arteries and their branches, along with the vertebro-basilar system, were filled with red silicone dye. A well-opacified blue silicone dye was seen in the cerebral veins and dural venous sinuses. A favorable consistency of the brain, without features of putrefaction, was observed. The brains were devoid of any disagreeable odor and could be dissected for prolonged periods.

**Conclusion:** Self-curing silicone dyes injected in the cerebral vessels enhanced the educational potential of the human cadavers, and served as a useful tool for the understanding of neurosurgical anatomical features, and learning various surgical approaches.

## STUDENT-TO-STUDENT TOURS AND THE MUSEUM OF PLASTINATES: ENGAGING THE NEXT GENERATION OF HEALTHCARE PROFESSIONALS AND THE PUBLIC

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**Introduction:** The Student-to-Student (S2S) program was launched in March 1986 by happenstance after three medical students showed a plastinated heart to a group of primary school children. Through this outreach program the medical students would bring wet specimens into community schools for the educational benefit of elementary and secondary students. The medical students agreed that this experience not only benefited the community schools, but also helped medical students to learn speaking skills and gain confidence in meeting the public. The S2S program was very successful but the excessive number of requests from the community schools made the coordination and availability of the medical students impractical. The solution was to bring the school children to meet the medical students on the medical college campus. Until 2013, medical students provided a presentation to the school groups in the gross anatomy lab, followed by a demonstration of select plastinated specimens. With the creation of the Interactive Museum of Anatomy and Pathology in 2013, the medical students were provided with a high-quality venue for the delivery of more structured tours. The Plastination Museum was created to provide valuable educational resources not only for the University of Toledo students in healthcare and related disciplines, but also for the public at large. It was also created with the purpose of educating students in the Toledo school districts and vicinity. The Museum is located in the College of Medicine, Health Science Campus, at the University of Toledo. The Museum provides a dynamic study and teaching space. The Museum was named after Dr. Liberato DiDio, former chairman of the Department of Anatomy, and Dr. Peter Goldblatt, former chairman of the Department of Pathology. The establishment of the Museum and its use will be discussed.

**Materials and Methods:** The “construction” of the Plastination Museum started 20 years ago when several plastinated specimens were created as a resource of didactic material to advance the teaching mission of the department of anatomy. Monies received by the plastination laboratory’s specimen preparation services (for other institutions) provided funding for the project. The original project budget rationale involved the following: relocating the plastination lab to free up space for the museum, renovating the old space with painting, installation of an acoustic ceiling and tile flooring, and the purchase of new wooden/glass display cabinets. The museum was constructed in three months and now houses approximately 300 specimens comprising the anatomical and pathological collections of the College of Medicine. Each cabinet was divided according to function (digestion, breathing, circulation, filtration, control, support, development, and comparative). Each cabinet has an android tablet containing explanations of each specimen, thereby providing a self-guided tour.

**Results:** The space allocated to the Museum, even though small, has been used appropriately by medical students and other healthcare students and professionals. Thousands of high school students from the Northwest Ohio and southern Michigan area have toured the Museum through the S2S Program. The S2S educational outreach program is organized by 1st and 2nd year medical students, who coordinate the Museum tours. Since 2013, the number of community students touring the Museum each year has increased as follows: 1,291 (2013-2014), 1,375 (2014-2015), 1,707 (2015-2016), 1,981 (2016-2017) and 2,507 (2017-2018).

**Conclusion:** The Plastination Museum was created with a limited budget but has proven to be an excellent method for housing the anatomical and pathological collections of the College of Medicine in a single location. The Museum has also proven to be an enormous asset to educate community students and the general public on normal human anatomy and diseases.

## NEW PERSPECTIVES IN PRACTICAL LESSONS USING PLASTINATED PARASITES IN VETERINARY DEGREE

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**Introduction:** Plastinated specimens have been used as an education tool in different subjects, mainly related with anatomy. However, few references exist about the plastination of parasites, most of them about the necessity of changes in conventional plastination protocols.

**Objectives:** The goal of this study was to evaluate the use of plastinated parasites as an innovative teaching and learning tool in parasitology practicals.

**Materials and Methods:** Seven different species of plastinated macroparasites were used: arthropods (*Oestrus ovis*); nematodes (*Parascaris equorum*, *Ascaris suum*, *Macracanthorhynchus hirudinaceus*) and platyhelminthes (*Fasciola hepatica*, *Dicrocoelium dendriticum*, *Taenia* sp.). A set of 89 students were involved, all of them assessed for previous background on the subject before any contact with the parasites. The experimental group of students used plastinated specimens in the practicals, whereas the control group used conventional wet specimens (preserved in ethanol and formaldehyde). After each practical session both groups were assessed with the same evaluation and also had the opportunity to score their level of satisfaction with the material (plastinated or not).

**Results:** The scores relating to knowledge and satisfaction after practical sessions did not show statistically significant differences between both groups ( $p>0.05$ ). Most of the students in the experimental group highlighted the facility of handling the plastinated specimens.

**Conclusion:** Plastination can be used to replace the traditional wet parasites in the practical sessions of Veterinary Parasitology without a decrease in the students' performing and evaluation scores. As has been already demonstrated in other subjects such as Anatomy, this is an effective way of avoiding the traditional use of wet, irritant, toxic and even carcinogenic chemicals in Parasitology.

## ACCESSORY LIGAMENT OF THE DEEP DIGITAL FLEXOR TENDON IN THE HORSE FORELIMB. A FLUORESCENCE STUDY WITH E12 PLASTINATED SECTIONS

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**Introduction:** The accessory ligament of the deep digital flexor tendon (AL-DDFT) is important for the correct function of the horse forelimb. Recently, a lateral fibrous lamina, binding the AL-DDFT to the superficial digital flexor tendon (SDFT) has been described by ultrasonography and MRI (FL-AL-DDFT). The main goal of this study was to describe its topographic anatomy by E12 plastinated serial cross-sections. 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 sectioning, from macroscopic to microscopic levels. The absence of manipulation and decalcification ensures that the topography of anatomical structures is unaffected. The removal of fat tissue allows connective tissue, blood vessels, and nerves to be identified quite clearly, without suffering any manipulation.

**Materials and Methods:** Ten forelimbs from two adults and four yearlings were used. Dissection techniques and epoxy (E12) transparent plastinated serial cross-sections were used to study in detail the topographical relationship between the AL-DDFT with the SDFT. The thickness of the sections was 2 mm. Plastinated sections were scanned with 1200 dpi resolution. Sections were studied under a Leica stereoscopic microscope. Plastinated collagen tissue had endogenous autofluorescence (488-nm excitation). Differentiation among fibers was based on their anatomical distribution and florescent intensity. A Nikon confocal scanning microscope was used to observe the plastinated sections. Thickness frame of the optical sections used was 15 – 20  $\mu\text{m}$  and 10X.

**Results:** At microscopic level the FL-AL-DDFT appears as a fibrous band in close association with the synovial membrane of the common digital flexor *vaina synovialis* (CDFVS). The fibrous connective tissue includes some capillaries and melanin pigment, and is likely to be covered by synovial epithelium at inner and outer sides. Thus, all along its proximo-distal projection, the FL-AL-DDFT may establish a lateral compartment in CDFVS which has not been described in detail so far. Further studies at higher (microscopic) magnification are required.

**Conclusion:** In addition to a mechanical bond between the AL-DDFT and the SDFT, the FL-AL-DDFT may be relevant for the synovial compartment between the DDFT and SDFT, which must be considered in all the pathologies affecting the function of the flexor component of the distal thoracic limb. Further studies are required, to further characterize the anatomical features of the FL-AL-DDFT depending on the age, breed, or different types of lameness.



## INFLUENCE OF SILICONE VISCOSITY IN TISSUE SHRINKAGE DURING THE PLASTINATION IMPREGNATION PHASE

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**Introduction:** Impregnation is the most important step in the plastination process, and is decisive for good specimen quality. The viscosity of the polymer is an important variable in the level of shrinkage observed during the impregnation process. Because shrinkage is one of the disadvantages of the technique, several researches seek ways to circumvent or reduce this retraction. The objective of this work was to test the influence of the viscosity of three different silicones on the shrinkage during the forced impregnation process at different temperatures.

**Materials and Methods:** For this experiment, 24 bovine kidneys were used, which were previously fixed in 10% formalin for one month, and were then organized into 2 groups: 12 for plastination at room temperature (RT) (25° C) and 12 for plastination at low temperature (-15° C). The silicones used were: Polioplast 1 (P1) and Polioplast 10 (P10) (Polisil Silicones Ltd.) and S10 (Biodur). Statistical analysis was performed using one way ANOVA followed by Duncan's post-hoc test or by two way ANOVA. The level of significance was considered  $p < 0.05$ .

**Results:** Comparing the three silicones of different viscosities, P10 showed the greatest shrinkage when used in the impregnation, with a mean of 39% at room temperature and 42.5% at low temperature. This is due to the fact that it has the approximate viscosity of 1250 mPas at 20° C (value supplied by the manufacturer), that is, the highest viscosity among the compared silicones (2.5X more viscous than S10). It is known that the higher the viscosity, the slower the speed at which the fluid moves, thus presenting a greater difficulty of penetrating the tissues. In the impregnation with P10, the shrinkage was much more evident at low temperature. Silicone P1 presented the best results, since the mean shrinkage was 4.75% at room temperature, and 15.5% at low temperature.

**Conclusion:** Polisil® P1 silicone appeared to be a good alternative to Biodur® S10 silicone, since it has a viscosity of at least 4X at room temperature, and 3X at low temperature, making it a viable substitution silicone of reference, since its cost of commercialization is more accessible in Brazil.

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## PRINCIPALS OF THE E12 TECHNIQUE

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The E12 technique was designed to preserve transparent body sections. Some technical aspects will now be emphasized regarding the E12 protocol.

### Sectioning of specimens:

Fresh or fixed specimens can be used. Vascular injections should be with epoxy instead of latex or silicone. The specimen should be frozen at the lowest possible temperature, at least -70 or -80 °C before, to obtain the transparent sections. Sections of 1.5-3 mm thickness are made with a band saw, and it is recommended to use dry ice or liquid nitrogen. The use of -40 °C acetone during the cleaning of the sawdust prevents sections from thawing.

### Dehydration by freeze substitution and defeating:

Dehydration of sections is carried out with cold acetone. Moreover, it is necessary to remove fatty tissue to get the highest transparency. This is done with several baths in acetone or methylene chloride at room temperature.

### Forced impregnation:

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

### Polymerization:

Polymerization must be done immediately after finishing the impregnation, to prevent polymerization in the impregnation chamber. Impregnated sections are introduced into flat glass chambers surrounded by E12+E1. The polymerization agent is the temperature: 40 °C for 4-6 days, combined with the hardener E1. There are alternatives to the flat glass chambers like the sandwich method, which saves time and is easier.

## RESULTS OF A RECENT SURVEY ABOUT ACTIVE PLASTINATION LABS

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This survey was addressed to the plastination community registered in the database of the ISP, and the historical list of participants in the workshops held in Murcia (Spain). The survey was open for a month (May 2018), submitted to 592 people (academics and technical staff), and answered by 62. Results showed that most participants were academics (61%), males (61.3%) and currently active in plastination techniques (74.2%). Around 66 % were members of the ISP, and at least 50% of them had been involved with plastination for a minimum of 5 years. However, 40 % of the participants never attended ISP conferences, or just once (20%). Plastination is mainly carried out in anatomy labs (36.8% human, 23.2% vets), but also in other venues or with other materials: museums (15.8 %), zoology labs (8.4%), fungi, flowers, etc. There is still a great interest in plastination workshops, especially those focused on advanced topics such as vascular injection, plastination of hollow and flexible organs, brains, clinical applications, and the research potential of plastination. Other suggested topics were: different methods of sectioning and casting in sheet plastination, brain reconstruction with P40 slices, staining of ultrathin epoxy slices, typical mistakes in plastination, and plastination for educational purposes. In conclusion, there is a need to define a strategy aimed at improving the coupling of the active plastinators with the ISP; plastination is quite well consolidated in non-anatomical fields; and there is an important potential for workshops focused on advanced topics in plastination.

## A WORKFLOW UTILIZING PLASTINATIONS FOR VISUALIZING AND ANNOTATING X-REALITY ANATOMICAL MODELS WITH HEAD MOUNTED AND CONSOLE BASED TECHNOLOGIES

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**Objectives:** Plastinated anatomical specimens are very useful as educational instruments. However, utilization in self-directed learning activities remains problematic since plastinations cannot be easily annotated. Augmented reality (AR) may provide a novel method to enable visualization and annotation of plastinated specimens. The purpose of this study was to develop an annotation system within an AR environment facilitating the use of labeled plastinated specimens.

**Materials and Methods:** A plastinated heart was imaged using a photogrammetry system. The digital model was polished using Z-brush software and then imported and processed using Vuforia software within the Unity3D engine. The 3D digital model of the plastination was converted into target points that recognized and tracked the heart in real time. Annotations could be added directly onto the tracked object within the Unity environment. In conjunction with a smartphone or head-mounted display (HMD) such as the Microsoft HoloLens, plastinations could be supplemented with annotations or additional contextual information within a text box display. The annotated virtual model can also be used for X-Reality applications as well as 3D printing.

**Results:** Results showed tight correspondence between the heart and associated annotations. The student could physically handle the plastinations while simultaneously visualizing the annotations and supplemental text box information. Qualitative comments among students indicated that they could utilize the models independently or in small groups, facilitating independent learning.

**Conclusion:** While such workflows were originally meant for CAD models and gaming, our application of photogrammetric methodologies enable real world plastinations to be overlaid with virtual assets and information. This AR tool in conjunction with plastinated resources may also be useful in clinical training sessions or museum displays. Work is being directed at developing quantitative tools to assess the educational usefulness of AR annotated plastinations.

## INFLUENCE OF THE TEMPERATURE ON THE VISCOSITY OF DIFFERENT TYPES OF SILICONE

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**Introduction:** The term silicone, or polysiloxane, describes mixed polymers of organic and inorganic materials, whose crude formula is  $[R_2SiO]_n$ , where 'R' are organic groups such as methyl, ethyl and phenyl. The main silicone used in the plastination process is polydimethylsiloxane (PDMS), referring to linear polymers, where the organic radical is methyl. One of the main external factors influencing the viscosity of a silicone is the temperature. The objective of this work was to test the influence of temperature on the viscosity of three silicones of different molecular weights (Biodur® S10, Polisil® P10 and P1), commonly used in the plastination technique.

**Materials and Methods:** For this study, the RheolabQC model rotational rheometer was used to measure the dynamic viscosities of the chosen polymers at the following temperatures: -5, 0, 5, 10, 15, 20, 25, 30, and 35° C. From the 9 measurements of viscosities obtained from each sample, a viscosity vs. temperature graph was constructed. The equation of the dynamic viscosity curve of each polymer was analyzed.

**Results:** Poliplast® 1 silicone had a much lower viscosity compared to other silicones (about 80 mPa.s at 25° C and 550 mPa.s at -25° C). Poliplast® 10 silicone presented the highest viscosity of the polymers analyzed (approximately 1180 mPa.s at 25° C and 3730 mPa.s at -25° C). The Biodur® S10 silicone showed an intermediate viscosity (about 410 mPa.s at 25° C and 1500 mPa.s at -25° C). The different viscosities found in the tested silicones are determined by the degree of polymerization. The larger the silicone chain (P10> S10> P1), the more intermolecular bonds are made with adjacent molecules and thus the less fluidity of the chain.

**Conclusion:** We conclude that Poliplast® P1 silicone presented the best physico-chemical characteristics of the tested silicones for plastination, because it has high fluidity and low viscosity. It is noteworthy that the viscosity of Poliplast® P1 in cold impregnation temperature (-15° C) is still lower than the viscosity of the Biodur® S10 (control) at room temperature (20-25° C). We also conclude that knowledge of the intrinsic and extrinsic physicochemical characteristics of the silicone, and its dynamic viscosity is helpful in choosing the ideal silicone for use in the cold or room temperature plastination techniques.

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## ENHANCING EFFECTIVE AND INNOVATIVE LEARNING IN ANIMAL ANATOMY DAF200

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Animal science consists of three major disciplines, namely: animal physiology, animal breeding, and genetics and animal nutrition. Animal anatomy and physiology (DAF200) is presented as a year module where practical sessions make up an important part of presentation and learning, as students need to observe and experience hands-on the texture, conformation, and anatomical organization, including splanchnology and topography of the animal body, for an improved understanding. Furthermore, reproduction physiology is presented on a third-year level, and students need to handle the reproductive organs before techniques such as artificial insemination can be practised on live animals. Approximately 120 and 75 students annually need to be taught in the anatomy and reproduction physiology modules, respectively.

In the past, dissected material was preserved in formalin, posing health hazards for both lecturer and students. This has been replaced with dissection of fresh cadavers for the past decade, but this practice has become costly, and has animal welfare implications, which can be regarded by some as unnecessary slaughtering and a waste of used carcasses. The use of fresh material may also pose other potential health risks to students and lecturers, such as Rift Valley fever or brucellosis.

In 2017, the Department of Animal & Wildlife Sciences decided to decrease the number of practical sessions on slaughtered sheep, due to costs and concerns with animal welfare and health risks. This has implications for effective learning outcomes, which led to the investigation of alternative methods to enhance future teaching and learning.

A project is underway where the different organs will be plastinated to be used during practical sessions. To enhance teaching and learning, plastinated organ specimens and even complete cadavers have become commonplace in human and veterinary faculties. In this project the various models will be developed, for example for the complete digestive system and reproductive system. An Anatomy Model Teaching Room is envisaged, representing the anatomy sections required for the modules presented by the department of Animal and Wildlife Science.

The use of plastinated specimens holds a number of advantages, including more effective hands-on teaching, fewer health risks for students and lecturers, more cost-effectiveness in the long-term. It will provide a sustainable alternative for practical teaching of anatomy and physiology in the Animal Sciences.

**COMPARISON OF BACTERIA ISOLATED IN THE ANATOMICAL LABORATORY AND OTHER BIOMEDICAL LABORATORIES  
IN THE MEDICAL FACULTY OF MUHAMMADIYAH UNIVERSITY OF PURWOKERTO, INDONESIA**

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**Background:** Infectious diseases are still in the top 10 causes of death in the world. The laboratory is a specific environment for the development of infectious bacteria. The anatomical laboratory, as a cadaver preparation area, plays an important role in the development of bacteria that can cause laboratory-acquired infection. Comparisons of bacterial isolates detected in the anatomical laboratory, and other biomedical laboratories, have not hitherto been widely known.

**Objectives:** To compare the bacterial isolates from the anatomical laboratory and other biomedical laboratories, at the Medical Faculty of Muhammadiyah University of Purwokerto, Indonesia.

**Materials and Methods:** The sampling technique used the settle plate method for environmental monitoring. Samples were taken with 5-point surface swabs from the anatomical laboratory and other biomedical laboratories (Histology, Microbiology and Clinical Pathology Laboratories) at the Medical Faculty, Muhammadiyah University of Purwokerto. Samples were grown on blood agar, nutrient agar and MacConkey agar, and then subjected to Gram staining and biochemical tests. Bacterial isolate data were analyzed descriptively.

**Results:** The total number of samples for this study was 20 samples. The bacteria identified were *Staphylococcus aureus*, *coagulase-negative staphylococci*, *Pseudomonas sp.*, *Enterobacter sp.*, *Vibrio vulnificus*, *Aeromonas hydrophila*, *E. coli*, *Enterobacter intermedium*, *Klebsiella pneumonia*, *Serratia fonticola* and *Streptococcus sp.* The Microbiology Laboratory had the highest contamination (63 colonies), while the Anatomical Laboratory was in third rank (17 colonies). The bacterial isolates found varied per laboratory. *Vibrio vulnificus* was the dominant bacterium in the Anatomical Laboratory (41.6% of 17 colonies), unlike the Histology Laboratory (*Enterobacter intermedium*), the Microbiology Laboratory (*Pseudomonas sp.*) and the Clinical Pathology Laboratory (*Enterobacter intermedium*).

**Conclusion:** Different bacterial isolates were found in each laboratory. The Anatomical Laboratory was dominated by *Vibrio vulnificus*, in contrast to the other three biomedical laboratories.

**COMPARISON OF BACTERIA NUMBER BETWEEN THE ANATOMICAL LABORATORY AND OTHER BIOMEDICAL LABORATORIES IN THE MEDICAL FACULTY OF MUHAMMADIYAH UNIVERSITY OF PURWOKERTO, BEFORE AND AFTER DISINFECTION WITH 70% ALCOHOL**

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**Background:** The laboratory is a specific environment for the growth of pathogenic and non-pathogenic bacteria. The anatomical laboratory, as a cadaver preparation area for medical students' practicals, can be a route for bacterial transmission. Disinfection is a useful process to reduce bacterial contamination, using chemicals such as 70% alcohol, to prevent bacterial transmission.

**Objectives:** To compare the number of bacteria between the anatomical laboratory and other biomedical laboratories, of the Medical Faculty of Muhammadiyah University of Purwokerto, Indonesia, before and after disinfection with 70% alcohol.

**Materials and Methods:** Duplicate samples were taken from the anatomical laboratory and other biomedical laboratories (Histology, Microbiology, and Clinical Pathology Laboratories) in the Medical Faculty of Muhammadiyah University of Purwokerto. The first and second samples were taken from the surface swab of each sampling point before and after disinfection. Samples were grown on blood agar, nutrient agar and McConkey agar, and then subjected to Gram staining and biochemical tests. Data of bacterial number differences before and after disinfection were analyzed using a paired t-test, while data of bacterial number comparison between each laboratory was analyzed by using an independent t-test and Mann Whitney test.

**Results:** The average number of bacteria before disinfection was higher than after disinfection. The difference between the two groups is very significant ( $p = 0.010$ ,  $p < 0.05$ ). The Clinical Pathology Laboratory had the highest bacterial contamination, and the Histology Laboratory had the lowest bacterial contamination. There was no bacterial number difference between the Anatomical Laboratory and the other biomedical laboratories ( $p > 0.05$ ).

**Conclusion:** The disinfection procedure is useful in reducing bacterial contamination. There was no significant bacterial number difference between the Anatomical Laboratory and other biomedical laboratories. The number of bacteria in the Anatomy Laboratory was not higher than the Clinical Pathology Laboratory and the Microbiology Laboratory.



## COMPARISON OF FIXATIVE SOLUTIONS AND THEIR INFLUENCE ON PLASTINATION PROTOCOLS IN HEPATIC, MUSCULAR AND ARTERIAL TISSUE

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**Introduction:** Plastination allows the use of specimens for histology purposes, but there are no references regarding specific results of different tissues. The objective of this study is to investigate if plastinated tissue samples from artery, liver and skeletal muscle can be used for routine histology, and how the fixation and plastination protocol used can influence the results.

**Materials and Methods:** Nine tissue samples from each organ (artery, liver and skeletal muscle) were fixed with three different solutions. Group I: 10% formalin; Group II: 2.5% formalin and Group III: Cambridge solution (an alcohol-based fluid containing phenol). Two samples from each group were plastinated with Biodur S10 technique, half of them cured, with the standard protocol, and the other half uncured (without curing). Sodium methoxide was used for the deplastination protocol. After deplastination, samples were paraffin-embedded for histology. Microtome sections were stained with H&E. The last sample from each group was a control sample, these were not plastinated, and processed following the classical histology protocol. During evaluation of results, each section was assigned an overall score based on the histological quality of the cellular components of the tissue. Sections were scored from 1 to 3 (1, good; 2, satisfactory/useful; 3, poor).

**Results:** Satisfactory sections were obtained from all tissues. The control sample from group I (formaldehyde 10%) resulted in consistently good quality of the tissue histology. Control samples from groups II and III resulted in consistently useful quality sections. In Group I, fixed with formaldehyde 10%, artery and skeletal muscle produced useful slides, and liver produced poor slides. In Group II, fixed with formaldehyde 10%, artery produced good slides, liver and skeletal muscle produced poor slides. For Groups I and II, fixed with Cambridge solution, artery produced poor slide quality, liver and skeletal muscle produced useful slides.

**Conclusion:** The tissues have different behaviors with different fixatives and plastinated protocols.

## HISTOLOGICAL EVALUATION OF SILICONE PLASTINATED SAMPLES PROCESSED UNDER DIFFERENT CONDITIONS.

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**Introduction:** Plastinated specimens can be used for light microscopy studies by means of deplastination techniques to obtain histological slides. The results of these deplastination techniques are susceptible of several improvements. One of them would be to minimize the negative effect of the fixation. On the other hand, previous authors have described a better preservation of the histological structure when the curing step from plastination protocol is avoided. Therefore, this study aims to assess the effect of several different fixation solutions, as well as the use of curing during plastination, on the histological structure of silicone-plastinated samples.

**Materials and Methods:** Twelve samples from each of the following nine organs: artery, esophagus, liver, small intestine, large intestine, skeletal muscle, nerve, pancreas, and trachea, were used for this study; in total 108 samples. Samples from each organ were divided into three groups (four samples each) depending of the fixative solution used.

**Group I:** samples fixed with formaldehyde 10%; **Group II:** samples fixed with 2.5% formaldehyde, and **Group III:** samples fixed with Cambridge embalming solution. After fixation, tissue samples were plastinated using the S 10 Biodur® silicone technique, however, each group was divided into two subgroups, depending on whether samples were cured or not, with two samples per organ in each subgroup. After the plastination process, tissues were deplastinated using sodium methoxide. After deplastination, specimens were processed for paraffin embedding. Sections were stained with routine H & E. The histology preservation was evaluated using a scale of 1 to 3, being: "1" the absence of morphological changes, good morphology; "2" minor morphological alteration; "3" intense morphological change.

**Results:** Tissues from groups I and III, fixed with 10% formaldehyde solution or with Cambridge solution and curing protocol, demonstrated the best histological results under light microscopy ( $P < 0.05$ ). Similar results ( $P < 0.05$ ) were observed in the slides obtained from uncured specimens of these two groups. Tissues from group II, fixed with 2.5% formaldehyde, cured or uncured, produced histological slides of level 3, with intense morphological changes, compared with groups I and III ( $P < 0.05$ ).

**Conclusion:** Plastinated specimens fixed with either 10% formaldehyde or Cambridge solution have a good histological preservation after deplastination. The curing step during the plastination procedure does not affect the histological results.

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## NEAR FIELD COMMUNICATION (NFC) DEVICES AND PLASTINATION: AN INTEGRATED TUTORIAL SYSTEM TOOL FOR SELF-DIRECTED LEARNING

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**Introduction:** The purpose of integrating Near Field Communication (“NFC”) with plastination is to provide students with a self-directed learning tool that offers intelligible flexibility for the study of anatomy. The objective of this learning tool is to offer students an interactive learning environment outside of the usual academic setting. This self-directed learning tool allows students to point at different parts of the plastinated anatomical specimen, which are tagged with smart chips, directing students to a web-page that presents a description of the specific anatomical muscle, nerve, or artery to which the NFC wand or smartphone is directed.

**Materials and Methods:** An application was designed to integrate the NFC reader, NFC tags, smartphones, Android tablet devices, and plastinates. The NFC is a set of standards for radio communication between tablets, smartphones and similar devices that allows communication with each other by proximity or touch. We used a vWand (SistelNetworks) in order to provide NFC connectivity for the Android tablet using Bluetooth connection. This wand allowed flexibility and uniformity within the system. Fifteen structures were identified in silicone- plastinated brain slices. Each anatomical description was entered in HTML format and stored as a webpage in the server. An NFC tag was attached to each structure and the URL web address of each structure was written into the tag using a vWand Pro Android app. When the tag was read by the vWand, an application launched the web browser containing a link to the webpage with the anatomical description of the structure. Similar techniques can be used with a smartphone by downloading an NFC Reader application. The smartphone will use its NFC reader to detect the NFC smart-chip tags on the plastinate. The smartphone will then open the URL containing the desired information within the NFC Reader application. The integration of the smartphone will allow for flexibility and convenience.

**Results:** The prototype consisted of 15 NFC tags implanted in multiple brain slices. Each tag when opened corresponded to a URL containing the description of the structure.

**Conclusions:** The NFC device is a platform for self-directed learning that integrates plastinates with digital technology, providing flexibility for the study of anatomy and pathology outside the usual academic settings. In addition, it provides an interactive environment, structure, and guidance to the student, and a powerful educational tool to promote meaningful learning through the integration of words, sounds and visuals.

## PLASTINATION OF BOVINE EYES AT ROOM TEMPERATURE

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**Introduction:** Bovine eyes are used for dissection in a wide range of anatomy courses. Use of plastinated bovine eyes will have long-term cost and time benefits for the institution and instructor.

### **Materials and Methods:**

#### Acquisition & Preservation

Bovine eyes (10) were obtained from slaughter within 1-4 hours from the time of death. The optic nerve and ocular muscles were exposed via dissection. Eyes were placed in an aqueous solution (40% ethanol and 10% formalin concentrate) for 14 days, and rinsed in a circulating water bath for 2 days.

#### Dehydration

An 18 gauge needle was inserted through the sclera into the posterior chamber approximately 1 cm posterior to the edge of the cornea. A second 18 gauge needle, attached to a 10 ml syringe, was inserted into the posterior chamber on the opposite side. Some vitreous humor (2-5 ml) was removed and discarded. Acetone (>98%) was injected into the posterior chamber via the syringe, positioned so that the acetone entered the eye from the bottom, with the first needle, at the top, as a vent. Acetone injection ceased when it began to spill out of the top needle. Acetone-filled eyes, with both needles inserted, were placed in a bucket of acetone (>98%) for 10 days, after which, each eye was reinjected/flushed with 10 ml of acetone (>98%) through the bottom needle, with old acetone escaping via the top needle. Eyes were placed into another bucket of acetone (>98%) for 10 days, repeating for a total of four cycles.

#### Impregnation

A mixture of silicone polymer (North Carolina Silicones, NCS-X) and 3% catalyst (North Carolina Silicones, NCS-III) was injected into the posterior chamber of each eye as acetone had been previously. Eyes were then submerged in the polymer-catalyst mixture and placed into a vacuum chamber, at room temperature. Pressure was decreased from atmospheric to 20 mmHg, over 5 days.

#### Dissection & Curing

Eyes were removed from the chamber and allowed to drain (2-7 days). The lateral surface of the posterior chamber was cut with a scalpel. The now opaque and gelatinous vitreous humor was delicately removed, leaving the retina intact and lens in place. Eyes were placed with the opening downward on an absorbent cloth, to allow the posterior chamber to drain (2 days), then placed inside an enclosure with vaporized NCS-VI (North Carolina Silicones) for 12 hours. Eyes were turned over and exposed to NCS-VI for another 12 hours, then removed.

**Results:** Dissection after silicone polymer impregnation of bovine eyes provided the best means to maintain placement of the retina and lens.

**Conclusions:** Plastinated bovine eyes are a useful tool in any anatomy course, due to the same general gross anatomy features as human eyes, but are large enough to easily visualize structural features. They are commonly available fresh from slaughter, and produce excellent plastinated specimens.

## PLASTINATION OF LARGE MAMMAL KNEES

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**Introduction:** Plastinated knees that retain the ability to flex and extend are a significant improvement over rigid cadaver knees or plastic models.

### **Methods:**

#### Acquisition& Preparation

Elk knees were obtained from a slaughterhouse. Connective tissue, remaining muscle, and most of the periosteum was removed to expose the ligaments of the knee. Tubing was placed between the medial and lateral collateral ligaments and their respective medial and lateral condylar surface of the femur. The tubing was to prevent the ligaments from shortening during the dehydration process.

#### Dehydration

After dissection, knees were placed in the first acetone bath (>98%) for 10 days, followed by three more changes of acetone (>98%) baths, each 10 days in duration.

#### Impregnation

Knees were submerged within in a mixture of silicone polymer (North Carolina Silicones, NCS-X) and 3% catalyst (North Carolina Silicones, NCS-III) and placed in a vacuum chamber at room temperature. Pressure was reduced incrementally from atmospheric pressure to 20 mmHg over 5 days.

#### Curing& Flexing

Knees were removed from the polymer-catalyst mixture. Gripping the femur and tibia, knees were made to flex and extend several times. Knees were flexed and extended daily for 5-7 days and then every 2-3 days over two weeks. After 3 months, knees were cured by exposure to the cross-linker (North Carolina Silicones, NCS-VI) overnight inside a sealed chamber.

**Results:** Flexion and extension abilities of large mammal knees were achieved by placing the fresh tissue directly into acetone without prior fixation with formalin, moving the knee daily after impregnation, and delaying the curing process by several months.

**Conclusions:** Large mammal knees are ideal for human anatomy joint lessons because of their large menisci and thick ligaments. The size of the elk knee is larger than human knees, and does not have a fibula. The health and integrity of the ligaments are superior to most of the knees from aged human donors.

## SMALL-SCALE, INEXPENSIVE ACETONE DISTILLATION AND PURIFICATION

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**Introduction:** In the initial stages of a plastination laboratory set-up, purchase of a solvent recycler for acetone may be as cost prohibitive as purchasing large quantities of acetone. A distillation apparatus assembled from typical laboratory equipment and standard organic ground glass jointware provides a cost effective way to recover pure acetone.

### **Methods:**

#### Vacuum Filtration

Dirty, used acetone from specimen baths was placed in a bucket in a freezer (<-15° C) overnight. The cold, dirty acetone was vacuum filtered to remove solidified lipid and specimen debris.

#### Fractional Distillation

The vacuum-filtered acetone was placed in the distillation apparatus. The fractionating column was fashioned from a 30 cm Leibig condenser loosely packed with wire from a stainless steel kitchen scouring pad. The vertical condenser connected via a vacuum adapter minimized the hood space required for the apparatus. Fitting the heating flask with a Claisen adaptor provided easy access for the addition of acetone with minimal disassembly. The heating mantle accommodated a 2000 mL round-bottom flask providing sufficient volume for processing on a convenient scale that required minimal attention. Condenser cooling was provided by circulating ice water with an inexpensive aquarium pump.

#### Purification

Distilled acetone was placed into 4 L flasks with 1 L of 3 Å molecular sieves, and left for 12-24 hours.

**Results:** The three-step process from water-fat-debris-containing acetone to >99% pure acetone took a total of 2 days for 6-8 L. Fractional distillation produced approximately 1 L per hour of acetone (96-98% purity) and >90% recovery of available acetone. After exposure to the molecular sieves, acetone achieved >99% purity.

**Conclusions:** This method produces 6-8 L of pure acetone (>99%) per day, to accommodate the smaller volumes, cost-constraints, and space limitations of a small-scale plastination lab.

**SOFT PLASTINATION METHOD FOR PREPARATION OF FLEXIBLE TEACHING MODELS**

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At the college of veterinary medicine, University of Basrah, the ambition was to produce low-cost educational anatomical specimens, using local materials that are easy to use and inexpensive, that are useful for dissection, and reduce the formalin exposure hazard. In order to confront these problems, a new soft plastination method was modified to obtain flexible, clean, curable, odorless, portable and non-toxic specimens that can be kept for long durations without deterioration. The advantages of this process are that it can be managed at room temperature by local inexpensive materials, and it can be accomplished with little experience in co-operation with the silicone plastination method. Students found the use of soft plastination specimens in the dissection room to be helpful in anatomical education, and it is a good and enjoyable method that improved understanding of anatomy, biology, embryology, and their related sciences.

## COMPARING EPOXIES BIODUR® E12 AND E12A FOR SHEET PLASTINATION

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In this study, epoxy resins BIODUR® E12 and BIODUR® E12A were compared with regard to their use in sheet plastination. While E12 is a well-established epoxy resin, E12A has been developed recently in order to obtain an epoxy especially suited for the flat chamber method.

Slices of approx. 3 mm thickness were plastinated using BIODUR® E12 or E12A in combination with hardener E1. The plastinated slices were cast either in a flat chamber or between sheets of polyester foil for the drain (sandwich) method. Results illustrate that the mixture of E12A, plasticiser AE21, and hardener E1 shows a viscosity of less than 1000 mPa\*s during a time span of at least 30 hours at room temperature. E12A therefore, is perfectly suited for the flat chamber method. On the other hand, E12 with its higher viscosity, is better suited for the sandwich method, because of its low tendency to allow for air entering the interstice between the slice and the polyester foil. Visual inspection showed that both E12 and E12A, yielded transparent plastinated slices of comparable, excellent quality.



## COLORING MUSCLE TISSUE FOR PLASTINATION

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**Introduction:** The visual appearance of the plastinated specimens is a very important factor to obtain an ideal product, which conserves the original appearance of the specimen. The skeletal muscle tissue comprises approximately 40% of the total body, being always very much in evidence in anatomical preparations. Therefore, the objective of this work was to test the staining and selectivity of different histological dyes (acidic and basic) by muscle tissue for use in the plastination technique.

**Materials and Methods:** Skeletal muscle tissue from carcasses of rats destined for disposal was used for staining. Each test dye was placed in 10% buffered formaldehyde solution, totaling a final volume of 300 mL of dye solution. The following dyes and their respective amounts were employed: ferrous fuchsin solution (7.5 mL), floxin B (0.0051 grams), safranin (0.0060 grams), Masson's trichrome solution (7.5 mL), and control (no dyes) for staining during the fixation step (30 days). After that, the tissues underwent the plastination process. Microscopy of stained tissues (prior to plastination) was also performed to evaluate the selectivity and adherence of dyes in tissues.

**Results:** All dyes had affinity for muscle tissue and did not stain underlying tissues. However, the affinity of Masson's trichrome was also observed in epidermis with microscopy analysis. It was possible to distinguish more easily the stained muscle tissue from the other tissues. The dyes that presented the best results and selectivity were those of acidic character (Masson's trichrome and floxin B), since they did not undergo the metachromasia phenomenon (color change of basic dyes in biological tissues). Among these, the most promising was the Masson's trichrome, since it more closely approached the actual color of muscle tissue *in vivo*.

**Conclusion:** It was verified that the coloration of the skeletal muscle tissue of anatomical pieces helps in the differentiation of tissues, such as the conjunctiva, adipose and epidermis. Masson's trichrome allowed coloration closer to the real thing, showing it to be the most suitable for the technique.

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## ROOM TEMPERATURE SILICONE PLASTINATION

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**Introduction:** There are two silicone plastination techniques all over the world. The classical method (S10) was introduced by Gunther von Hagens in 1977. It uses a reaction 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 a non-reaction mixture silicone mix, 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-temperature 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, 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; the difference is in the impregnation and curing steps only. The RT impregnation mixture consists of 93% silicone and 5% cross-linker. That is not a reaction mixture, and so there is no need to keep it in a freezer. Molecular weight is about 500, and 12-second dynamic viscosity. The average shrinkage calculated for the tissue cores plastinated by the RT technique ( $16.2 \pm 1.49\%$ ) was 1.5 times less than by the cold method ( $p < 0.05$ ). The total duration of the impregnation and curing stages of samples for the RT plastination was found to be 1.54 times shorter than that of the 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 the RT technique.

**Conclusion:** The specimens plastinated with the RT technique were 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, parts of the body, fetus, fur/hair/feather-covered specimens, reptiles & fish, as well as for long time formalin-fixed specimens, for archaeological and fossil objects. A room temperature plastination laboratory is more economical to set up than the cold-temperature system.

## E12 PLASTINATION FOR RESEARCH INTO STENTED CORONARY ARTERIES

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**Introduction:** Stenting of coronary arteries is the most promising method of myocardial revascularization for ischemic heart disease. At the same time, detailed visualization of the metal stent inside the coronary artery, and study of the relationship of its elements to the arterial wall in histological specimens, is not possible. Use of a special technique of epoxy plastination for stented coronary arteries contributes to new treatment methods for one of the most common cardiovascular pathologies.

**Materials and Methods:** A portion of the heart wall with an implanted stent was dehydrated in cold acetone at -25° C. After completely replacing the water, the preparation was immersed in mixture of low-viscosity E12 epoxy resin and hardener in a ratio of 20: 1, and impregnated in a vacuum chamber at 30° C, decreasing pressure gradually until release of acetone bubbles ceased. The heart wall was then taken out of the resin, and left for 7 to 10 days at a temperature of 40° C until it was completely cured. Tissue was removed around the stented artery by grinding, or the specimen was cut with a diamond saw in 0.5 – 1 mm sections. The sections were placed in polymethylmethacrylate flat chambers, and filled up with a composition of E12 epoxy resin and hardener in the ratio of 10: 1. After hardening of the resin, the transparent specimens of stented arteries were scanned at 600 or 1200 dpi, or were microscopically examined up to 20X magnification.

**Results:** Plastinated E12 slices provided good anatomical details down to the microscopic level. The proposed method is fine for detecting changes in the geometry and morphology of stented lesions of coronary arteries. It permits good visualisation of the contact of the stent with the inner vessel wall and atherosclerotic plaques, and it easily provides measurements and statistical analysis. In comparison with radiography, this method makes possible evaluation of the topography of the metal implants in arterial bifurcations, and it demonstrates the degree of deformation of the atherosclerotic plaque after stenting. Hard transparent sections with whole stents retain transparency and can be cut transversely for more detailed examination.

**Conclusion:** Adequate impregnation of the heart wall is achieved by using low-viscosity epoxy resin, and heating the impregnating composition, which increases the fluidity of the resin, and enabled impregnation of specimens with thickness up to 25 mm. This method can be applied for clinical research for development of new methods of stenting of coronary arteries, and for the study of other organs with implanted metal constructions and devices.