

# Plastination Technique in Laboratory Rats: an Alternative Resource for Teaching, Surgical Training and Research Development

Técnica de Plastinación en Ratas de Laboratorio: Un Recurso Alternativo para la Enseñanza, Entrenamiento Quirúrgico y Desarrollo de la Investigación

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**SUMMARY:** Today, alternatives methods are developed for the use of laboratory animals for teaching, research and surgical training. In our work we present a novel alternative to the use of rats, by developing a technique of plastination at room temperature. High-quality rat preparations from the anatomical dissection point of view were obtained, in order to indefinitely preserve them dry, the thoracic and abdominal organs conserve its natural volume and shape, maintaining their texture and color. No odors or hassles and toxic vapors of conventional preserving agents were found. This technique allows the collection of dry, completely biosafe and durable specimens in a short time and with excellent quality. Plastination in laboratory rats complements undergraduate and postgraduate anatomy studies perfectly. Also, radiology and surgery may benefit from this technique.

**KEY WORDS:** Plastination; Room temperature; Laboratory animals; Rats.

## INTRODUCTION

Working with animals in a laboratory requires a trained and experienced animal care staff, with knowledge of biosafety regulations, in order to handle them correctly, as they become a biological reagent. Well-trained and experienced personnel in both practical and theoretical aspects are essential to fulfilling with international ethics regulations regarding laboratory animal handling, including Russell and Burch's three R's (replacement, reduction and refinement). Every teaching or research institution must develop an internal certification, with normalized operating procedures and satisfying ethical and laboratory good practice principles, to develop a responsible attitude towards animal experimentation.

It is essential to know that specimen fixation is a risky process and that laboratory personnel should be trained in

handling hazardous material. Plastination of laboratory animals allows for safe specimens but also significantly reduces exposition of laboratory personnel and graduate and postgraduate students to toxicity derived from preserving specimens in formalin or other preserving substances.

Plastination is an anatomical technique used to preserve biological material with educational and training purposes. It was developed by Professor Gunther von Hagens in Heidelberg, Germany, in 1977 (von Hagens, 1979; Bickley *et al.*, 1981; von Hagens *et al.*, 1987; Ottone, 2013). This technique consists in substituting tissue fluids (water and lipids) by polymers. Specimens do not lose apparently normal texture and color features, thus resulting in odorless, dry specimens that are highly durable. After the specimen has been impregnated, it becomes much more stable than frozen,

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dehydrated, or paraffin-treated specimens. Additional benefits include keeping texture and cellular identity at a microscopic level (Tianzhong *et al.*, 1998; Henry, 2007; Raof *et al.*, 2007).

Its use in animal models, allows overcoming some limitations with human parts and their availability or ethical and legal considerations involved (Riederer, 2014). Plastinated rodents may become an excellent resource for teaching as they allow consulting, practicing gripping and handling, acknowledging anatomical relations and features, establishing reference points for surgical maneuvers, endoscopic training, develop autopsy protocols, among other purposes (Stuart & Henry, 2002; Latorre *et al.*, 2007).

The objective of this paper was to describe and promote a room temperature plastination technique in rodents as a resource in teaching and research, in order to facilitate training of personnel involved in research with laboratory animals.

## MATERIAL AND METHOD

Five Wistar rats of similar ages and weights were used. These were obtained from the animal facility located at Department of Histology and Embryology, Faculty of Dentistry, University of Buenos Aires (the protocol was approved by the Bioethics Committee of the Faculty of Dentistry, University of Buenos Aires). The animals were kept under a 12/12-hour light/dark cycle, with a temperature of 19–23°C, relative humidity of 50%. Drinking water and food were provided ad libitum. The rats were taken care of, and received analgesia, anesthesia, and euthanasia procedures following international human care of laboratory animals and recommendations of the animal protection code used at Faculty of Dentistry's animal care facility.

The rats were euthanized by intracardiac injection of 0.2 ml of pentobarbital sodium euthanistic diphenyl hydantoin (Eutanyle, Laboratory Brower) with the animal previously anesthetized with a combination of xylazine 2% (Kensol, König Laboratory) and 5% ketamine (Ketamine 50, Holliday Laboratory) at a dose of 5 mg/kg xylazine and 50 mg/kg ketamine.

After euthanasia, we proceeded to dissection. The animal was placed in a supine position and a longitudinal thoraco-abdominal incision in the midline was performed. Then, two incisions in the upper and lower ends, perpendicular to the longitudinal incision was made, allowing a

greater opening of the thoracic and abdominal cavities, in the specimens of both sexes.

The samples were first fixed in a 5% formaldehyde solution and later placed in a pool with the same proportion of formalin for one week in order to prevent the specimen's putrefaction and autolysis, killing all bacteria and certain proteins that may remain after dehydration and lead to decomposition. Furthermore, this phase is basic because it makes the preparation solid and helps reducing shrinkage after plastination.

The plastination was performed at the Plastination Laboratory, Institute of Morphology J.J. Naon, Faculty of Medicine, Universidad de Buenos Aires, Argentina. Below, we describe the plastination technique developed:

**Dehydration.** This phase aims to replace the formaldehyde solution with an intermediary solvent, a water-miscible solvent, such as acetone. Dehydration at room temperature (20°C) lasted 2 weeks. For this, the sample was placed in a container of the same size, fully immersed in acetone, taking cover by about 2 cm from the sample. Then, the samples were subjected to 2 baths with 100% of acetone. Also, for the first bath could be used a greater than 90% concentration of acetone (reusing previous acetone baths is possible). The purity was measured with an acetometer. This stage at room temperature also allows the defatting of specimens.

**Forced impregnation.** This is the most important phase of the plastination process and should be carried out slowly. Forced impregnation extracts the intermediary solvent (acetone) from the cell's structure and the preparation's interstice and replaces it with silicone. This exchange process is due to the high vapor pressure of acetone against the low vapor pressure of the silicone-catalyst mixture, where the volatile intermediary in the preparation is continuously withdrawn by a vacuum pump, until it is completely removed, generating a pressure difference, allowing the polymer to enter into the preparation. In our case, the impregnation's speed is carefully adjusted by a controlled air addition into the vacuum pump via a bypass valve.

We have developed the forced impregnation in two phases: "active" and "passive". Active forced impregnation consists in generating vacuum in a continuous fashion, inside the vacuum chamber, through the action of a vacuum pump. The anatomic material is placed inside a compact-sized plastic container, custom-made for the preparation, to avoid wasting silicone (Fig. 1). The mixture in which the preparation is immersed includes generic silicone and generic catalyst / chain extender in a 100:1 ratio. The



Fig. 1. Forced impregnation of two specimens inside the vacuum chamber.

preparation should be completely immersed in the mixture, using elements that keep it under. The whole vacuum process is carried out at room temperature. The pressure should be reduced by closing the valve (vacuum increases) until bubbling is observed. When bubbling occurs, pressure should be maintained so the process occurs at that pressure; that is, to keep bubbling by partially opening the valve. Bubbling is acetone vapor leaving the specimen and the silicone-catalyst mixture occupying the spaces left. Accurately, reducing the pressure to achieve greater vacuum, to reach the final goal of 5 mmHg, is not performed continuously. Instead, the preparation is under “active” forced impregnation during 8 hours each day. Once these 8 hours are completed, the vacuum chamber’s valves are closed and the vacuum pump is switched off. The preparation is left inside the chamber, and the passive forced impregnation phase begins. This phase lasts until the next day when it starts again from the pressure at which the process was stopped before. Once the 5 mmHg is reached and bubbling stops, the forced impregnation phase will be finished.

**Draining.** The preparation is removed from the silicone-catalyst mixture and left to drain in the same container to remove the excess silicone. Using paper to contribute to remove the silicone excess is also possible, and in the case of hollow entrails paper is placed inside cavities of the specimen to absorb silicone excess and help it dry. This can take two days, and depends on the size of the preparation. The time spent to drain was 7 days.

**Positioning.** Once the preparation has completely drained, we proceed to position the specimen’s anatomical structures according to which section of the dissected region will be displayed. Positioning takes place at this time before the curing, when the specimen becomes hard and impossible to move.

**Curing (polymerization).** This process consists in the final curing and drying of the preparation. Silicone manages to stay inside the tissue. After removing the silicone excess, the

specimen is placed inside a curing chamber and is exposed to the gas cure (generic hardener). It is placed inside a container and using a water tank engine, the hardener, that is liquid, starts bubbling until it is gasified. This acts upon the preparation, aiming at achieving the final drying and hardening of the preparation. It is important that the preparation is placed inside the curing chamber with silica crystals, which absorb moisture and keep the curing chamber interior dry. So there are two curing stages: fast and superficial curing, which takes 2–3 days, by exposing the specimen to the gas cure. This process achieves the external drying of the sample. However, during 3 or 4 months, the second stage starts: prolonged and internal curing. The specimen should be kept inside a plastic bag to achieve the final polymerization at the inner section of the preparation. Once this process ends, the specimen should be kept and stored at room temperature, with minimal care requirements.

## RESULTS

The results are shown in Figures 2 and 3. The total time required to perform the technique was 32 days, divided as follows: 1 day for dissection, 14 days for drying, 5 days for forced impregnation, 7 days for draining excess silicone 5 days for polymerization of the specimens. In Table I, describes in detail the duration of forced impregnation.

All rats had the same size and weight, whereby the times of the technique were the same for all the specimens used. However, the time will vary in direct proportion to the size of the specimen, and especially during the process of forced impregnation. The larger the specimen, the higher the extent of impregnation time, extending in two or three days, always in the case of such specimens.

Drainage and polymerization times were also extended. The dehydration process is kept constant without changing by the size of the specimen.

Our proposal for an active and passive forced impregnation ensures the smooth incorporation of the mixture silicone-catalyst into the specimen, to avoid exaggerated and premature retraction of tissues, ensuring their original size and shape.

All rats were optimally plastinated, so we could get high-quality rats preparations from the anatomical dissection point of view in order to indefinitely preserve them dry, the thoracic and abdominal organs conserve its natural volume and shape, maintaining their texture and color. There was no difference in the final outcome by sex or weight.



Fig. 2. Specimens after plastination, ready to be used. Abdominal organs are displayed.

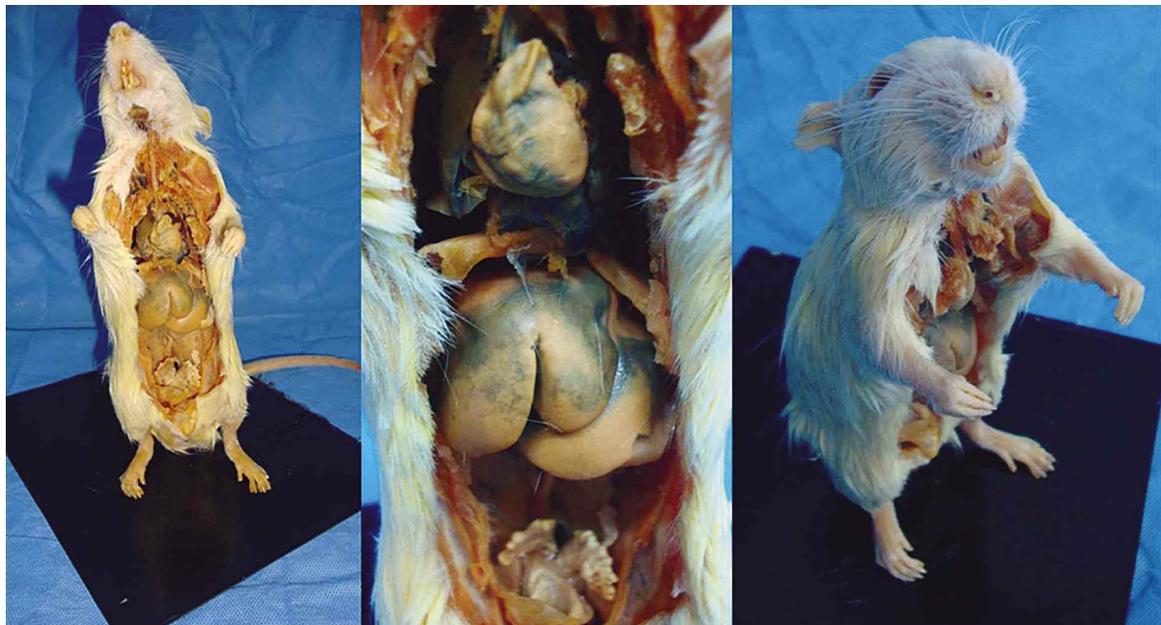


Fig. 3. Another specimen (female rat) after plastination, positioned so that can stay on two legs for proper display of thoracic, abdominal and pelvic organs.

Table I. Forced impregnation process. This stage lasts for five days, in which periods of active forced impregnation (8 h each) and passive forced impregnation are combined (12 and 24 hours depending on the day). In this way it is sought to bring the pressure inside the vacuum chamber of 760 mmHg to 5 mmHg, and in this final pressure, when the bubbles disappear, the forced impregnation process is over.

Days	Active forced impregnation (8 h)		Passive forced impregnation
1st day	---	---	760 mmHg (24 h)
2nd day	760 mmHg	460 mmHg	460 mmHg (16 h)
3rd day	460 mmHg	160 mmHg	160 mmHg (16 h)
4th day	160 mmHg	5 mmHg	760 mmHg (24 h)
5th day	---	---	760 mmHg (12 h)

No odors or hassles and highly toxic vapors of conventional preserving agents that are extremely toxic and irritating, such as formaldehyde and phenol were found. Manual handling and specimen resistance to handling are very convenient.

## DISCUSSION

The plastination technique using silicone at room temperature, described in this article, has allowed us to obtain high-quality specimens in a short period of time and at a low cost. This was achieved thanks to the local construction of the iron vacuum chambers and obtaining generic products, corresponding to silicone, catalyst and hardener. The lack of utilization of freezer not only cut the costs, but also contributed to the laboratory biosafety.

Handling biological material bear some risks regarding the specimen that will be preserved for dissection and anatomical examination, and the reagents used for fixating and preserving specimens. With this in mind, plastinated rodents are completely biosafe. This becomes essential if plastinated specimens are compared to samples preserved in the usual way. Plastinated samples are immersed in silicon, which is non-toxic and do neither release gases nor toxic substances. That is why they can be shown around in anatomy classes in pregraduate studies. The plastinated sample is a “friendlier” approach to cadaveric material. Visual impact of death on first-year students (quote authors) is less shocking than when specimens preserved in formalin are used, as formalin odor is not present, and plastinated specimens look more real.

The development of the plastination technique, as you may see in this work, achieves dry, odorless, durable, real, non-dangerous specimens that do not require maintenance and do not deteriorate with time. Another great advantage is that students and teachers may handle them without wearing gloves or following any safety measure. Museum specimens, preserved inside jars, are difficult / dangerous to manage. So are wet specimens preserved in formalin or in another preserving agent (Bickley *et al.*, 1987; Dawson *et al.*, 1990). This technique solves this issue.

Regarding maintenance of plastinated specimens,

there is no need to use large pools with preserving agents, freezers, vacuum containers (Baptista *et al.*, 1992). No other substances, techniques or devices are required. Plastinated samples are kept inside plastic bags and stored at room temperature (20–22°C).

Regarding dissection activities, some authors think plastination may replace dissection of specimens. We, as well as other authors (Latorre *et al.*; Rieder), do not agree with this statement, plastination is not a replacement for traditional guided dissection, but it does provide an additional learning tool to understand complex anatomy. We consider plastinated specimens are to be used as a complement to dissection, if it can be done. Plastination may become useful for dissection students: they may first study anatomy and dissection planes in plastinated specimens and then dissect bodies more easily and safely.

Medical schools have started to show plastination not only as a basic tool for teaching and learning but also as a training and research method in postgraduate studies. The potential in plastination lies in its capacity for preserving delicate structures (Valenzuela *et al.*, 2012), and its interrelations, including microscopic structures (Jones, 2002).

Besides this technique’s advantages regarding biosafety, highly durable and low-maintenance samples, we have developed a plastination technique at room temperature that can be performed at a low cost, using local products and readily available equipment that any institution may acquire (Ottone *et al.*, 2014). However, trained personnel are required to adequately carry out plastination.

In conclusion, plastination in laboratory rats complements pregraduate anatomy studies perfectly. Dry, odorless, completely biosafe and durable specimens are obtained. Anatomy, radiology and surgery may benefit from this technique. Postgraduate studies, for instance training in different surgical and dissection approaches, and morphological research activities also benefit from it.

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**RESUMEN:** En la actualidad, se desarrollan alternativas para el uso de animales de laboratorio para enseñanza, investigación y entrenamiento quirúrgico. En nuestro trabajo presentamos una novedosa alternativa para el uso de ratas, a través del desarrollo de una técnica de plastinación a temperatura ambiente. Se obtuvieron preparados de alta calidad desde el punto de vista de la disección anatómica, con órganos torácicos y abdominales que conservaron su volumen, forma, textura y color. Además, los especímenes carecen de olores y no emiten vapores tóxicos, debido a la ausencia de agentes conservantes convencionales. Esta técnica permite desarrollar especímenes secos de excelente calidad, completamente bioseguros y duraderos, en muy poco tiempo. La plastinación en ratas de laboratorio complementa los estudios de anatomía de pregrado y postgrado perfectamente. Además, las áreas de radiología y cirugía también pueden beneficiarse de esta técnica.

**PALABRAS CLAVE:** Plastinación; Temperatura ambiente; Animales de laboratorio; Ratas.

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