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Corrosion Casting, a Known Technique for the Study and Teaching of Vascular and Duct Structure in Anatomy

Inyección Corrosión, una Técnica Conocida para el Estudio y Enseñanza de Ductos y Estructuras Vasculares en Anatomía

Roberto Javier Rueda Esteban[1]; Juan Sebastián López McCormick[2]; Daniel Ricardo Martínez Prieto[3] & Juan David Hernández Restrepo[4]

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SUMMARY: Teaching and learning anatomy, as a process, has changed. Fresh cadavers were once used as a tool for the student to approach the human body in order to overcome theoretical knowledge and gain applied expertise. Today, techniques such as corrosion casting are known to be a more effective way of achieving optimal results with the students. This paper examines a method to apply this technique to an organ using different polymers. The concentrations for acrylic, epoxy resin, polyester resin, and room temperature vulcanization (RTV) silicone are described, as well as the corresponding diameter of the duct to be injected with each one. A variety of specimens obtained using this technique, their qualities and characteristics are presented. The results of using these procedures while involving the students, showed increased sense of responsibility, dedication and awareness, which led them to take the class more seriously and enjoy the process of learning. Additionally, the specimens left by the students will help future classes reduce the number of specimens needed.

KEY WORDS: Corrosion Casting; Repletion; Corrosion; Teaching Anatomy; Preservation Techniques.

INTRODUCTION

The concern for the conservation and preservation of the human body has been a constant issue since ancient times (Bustamante *et al*., 2007), but it was not until the 16th century that the preservation of corpses for educational and didactic purposes was first considered. Historically, hundreds of chemical substances (alcohol, tannic acid, mercuric chloride, arsenic, glycerin, formaldehyde, among others) have been used to avoid the natural putrefaction process to study the human body and to preserve cadaveric dissection as an essential activity in medical education (Korf *et al*., 2008). Nowadays, polymers as a method of preservation, are the first defensive line of the anatomists against this natural process. They not only avoid putrefaction, but also show more durability and specimens do not have to be disposed due to decay throughout the years (Bravo, 2006). Moreover, these specimens reduce or eliminate the biological risk present in the manipulation of cadavers treated with other substances.

Background. It is important to note that in anatomical education, the process of teaching and learning anatomy has been affected by many different factors that are not the topic of this document. However, these factors forced anatomists to search for new ways to maximize the student´s appropriation of anatomical concepts independently of the time dedicated to the course in the curriculums of different medical schools.

The accomplishment of dissection projects as practical activities, has shown to improve the learning process by motivating and engaging students (Tefera, 2011). Nevertheless, dissection is not a discipline on its own, but one of many tools used for didactic teaching of anatomy (Aversi-Ferreira *et al*., 2010). In fact, a combination of methods and activities have been recommended to increase the students learning opportunities (Tefera). Even though,

[1] Universidad de los Andes, School of Medicine, Assistant Professor and Anatomy Coordinator, Anatomy for Education Research Group, Bogota D. C. Colombia.
[2] Universidad de los Andes, School of Medicine, Student, Anatomy for Education Research Group, Bogota D. C. Colombia.
[3] Anatomy for Education Research Group, Bogotá D.C. Colombia.
[4] Universidad de los Andes, School of Medicine, Anatomy and Surgery Clinical Professor, Anatomy for Education Research Group Bogotá D.C. Colombia.

basic knowledge of anatomical dissection is necessary to achieve an integral learning of anatomy, students consider it to be better when it has a variety of resources (Dettmer *et al*., 2010). That's why in current anatomy curriculums, teaching is not only based on theory and practical dissection but also on the integration of many tools like multimedia and diagnostic images, among others.

The injection and corrosion technique has had mayor changes through time, but its goal has always been the same: to expose the void of any injectable duct. Records of its use can be traced back to the 1600', when Swammerdam, Boyle, Pecquet and Ruysch first made the technique widespread, with Swammerdam having the general adoption of the technique attributed to him (Narat *et al*., 1936). At its inception, the process of injection and corrosion had to start in vivo, to achieve good outcomes after washing with Ringer lactate, pre-mortem anticoagulation of the specimen (Tucker & Krementz, 1957) or the fixation of the specimen in formaldehyde during a certain time (Tompsett, 1959). Today, these steps are considered unnecessary or unethical.

A variety of materials have been used, from Frederick Ryusch's secret colored mass, of which the recipe is kept in secret, to Govard Bidloo's metal alloy containing bismuth and mercury, the evolution of the materials used has been staggering (Narat *et al*.). Nowadays the usage of polymers and resins is the rule due to the ease of handling, safety, good hardness when dry, and their characteristic of not contracting or altering their size compared to the original casting.

The process of injection and corrosion has proven not only to be successful for its anatomical use in education, but also for research. It permits the analysis of microvascular structure and its complex organization, as seen in Figure 1, even allowing three-dimensional observation (Minnich & Lametschwandtner, 2000), and promoting the correct interpretation of diagnostic images in medical praxis (Matamala *et al*., 1984). Even though it is a low-cost procedure used by many Universities around the world, there is no current literature that allows someone who is starting to practice this technique to understand the process, polymers, and how to use them. The objective of this paper is to describe this technique, and some of the polymers that can be used.

MATERIAL AND METHOD

The objective of injection and corrosion is to preserve the polymer matrix (internal conformation) of one or several ducts of an organ or even an entire body, then eliminating all the surrounding tissue, and finally obtaining a tridimensional polymeric specimen that shows the internal conformation of the initial piece, without any biological risk.

As can be inferred by its name, this technique has two phases: The first one consists of injecting a polymer inside the ducts that want to be shown. These might be arteries, veins, biliary ducts and urinary tract, amongst others. The second phase, corrosion, consists on the elimination of all the surrounding tissue, leaving only the matrix that has already been injected with the polymer. Both phases should be preceded by the dissection and preparation of the specimen. These phases and the necessary procedures to achieve them will be described, using as examples the best outcomes in our experience.

Preparation. It is important to point out that the injection process has shown improved results in fresh specimens. That means a specimen without any previous fixation or formalin injection, since these processes imply a hardening and retraction, mainly in vascular tissues, further complicating the steps in the process. A thorough wash with water for several minutes will remove clots and other debris, and improve the visualization of the structures of interest.

Depending on the structures being injected and the organ of interest, it is imperative to pursue a previous careful dissection of the main ducts to avoid damaging them or the surrounding tissue, because even though this tissue is going to be removed in the final phase of the process, it is important to keep the tridimensional structure intact and to avoid leakage of small terminal branches injured during dissection.

Injection

The stage of injection of the polymer in the ducts is commonly underestimated because of the simplicity of its definition, but it is the process that requires the most skill and complex processing of the technique, depending on the structure that is to be injected. It is divided into three steps:

Dissection and Identification: After a careful preparation of the specimen, it is necessary to release the ducts, which have been previously identified. Since the injected polymers can be tinted to improve the clarity of the final piece, a color code has been established to differentiate structures: red - artery, blue - vein, white - airway, yellow - urinary ducts and green - biliary conducts.

Isolation and Cannulation (Hill & McKinney, 1981): A process that has to be conducted very carefully in small vessels and ducts, because they can be easily injured to a

Table I. Concentrations, uses, and characteristics of different injectable materials.

Material	Components	Concentration	M.T. (MIN)	M.I.T. (MIN)	H.T. (HOURS)	Recommended Diameter Of The Duct That Is Being Inyected
ACRYLIC	Methyl methacrylate (LIQUID) / Self curing acrylic (SOLID)	1,2 GR/CC	1,5	2	2	6MM - 8MM
		1,0 GR/CC	2	6	4	4MM - 6MM
		0,6 GR/CC	5	12	5	SMALLER THAN 3MM
EPOXY RESIN	Component A (LIQUID) / Component B (LIQUID)	2 A / 1 B	10	20	4 TO 8	BIGGER THAN 7MM
POLYESTER RESIN	Resin (LIQUID) / Meck Catalyst Peroxide (LIQUID) / Accelerant (LIQUID, not used)	15 CC MECK / 1 KG RESIN	10	20	6 TO 10	BIGGER THAN 8MM
RTV SILICONE	Silicone (LIQUID) / Catalyst (LIQUID)	0,05 GR CATALYST / 1KG SILICONE	5	10	1 TO 6	BIGGER THAN 8 MM

(M.T.) Recommended Mixing Time, (M.I.T.) Maximum recommended Injection Time, (H.T.) Recommended Hardening Time before starting corrosion. Considering temperature and humidity conditions at the Anatomy Laboratory, Universidad de Los Andes, Bogotá D.C. Colombia.

point that renders the injection impossible. This is best done with a tube of the correct size and material that permits the injection of the polymer. This may vary depending on the duct that is going to be injected. If a tube that has the right diameter cannot be found, it is best to use a smaller one. If a tube with a bigger diameter is the only means available this can be utilized with wedge cuts in the terminal end that will be introduced in the duct (Fig. 2). This step must be done with extreme care.

After introducing the tube in the duct, it must be fixed in place to avoid any leaking of the material injected under pressure. This fixation is done with a transfixion suture, with alternating knots on each side of the duct. Preferably use multifilament 2-0 sutures or thinner, depending on the duct to be injected. This type of suture avoids knot loosening due to high pressures induced during injection, furthermore it can be used in any duct and the damage caused by the suture is minimal, which prevents the injected material leakage. In ducts with 5 mm diameter or wider, dental floss or hemp thread can be used. The purge can be performed by injecting each positioned tube with water or with hydrogen peroxide (H_2O_2 1-2%) in very wide ducts (mainly airways). This is necessary to eliminate clots, stones or any possible blockages that can interfere with the polymer's entry, facilitating the injection. If H_2O_2 is used it is important to wash with water afterwards because the foam produced can affect adequate injection and the compound itself can damage surrounding tissue.

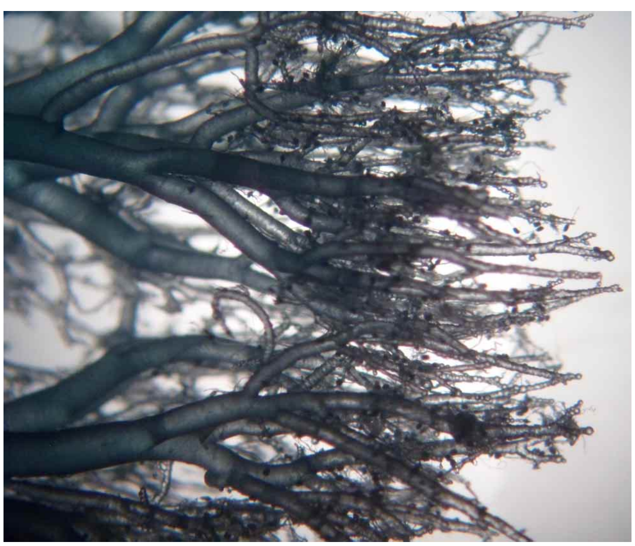

Fig. 1. Magnification using a stereoscope (40x) of a goat kidney injected with Methyl-Methacrylate through arterial vasculature.

Injection: After the isolation and cannulation of the structures, it is important to prepare the chosen polymer for the organ; the polymer that has shown the best results in our experience is acrylic. Other polymers that can be used with variable results are polyester resin, epoxy resin, RTV silicone and polyurethane.

After preparing the polymer it must be injected directly on each tube independently using positive pressure pumps. The pumping can be done with a compressor and a polymer container or manually with different sized syringes, depending on how much polymer is going to be injected. If one injection is not enough a clamp should be placed on the tube - remove the syringe plunger - refill the syringe and then remove the clamp and resume the injection immediately

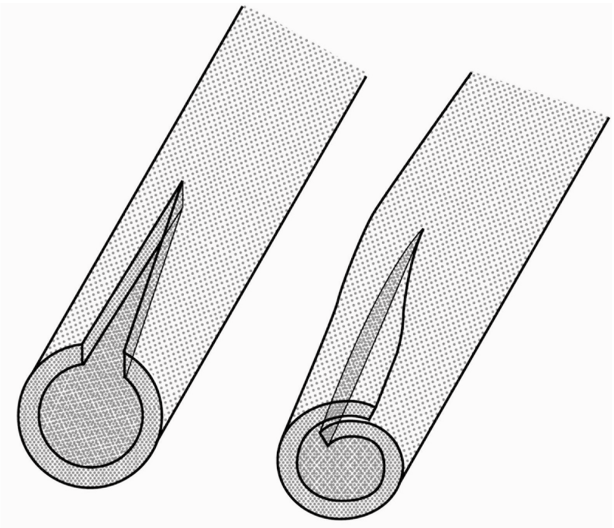

Fig. 2. Wedge cut needed to adapt tubes with larger diameters than the duct to be injected.

to avoid the formation of bubbles or polymer reflux. In the final step of this first phase, all the tubes must be sealed with different clamps (Kelly, Rochester, etc.) and then the polymerization process should be allowed to happen for the necessary time. To obtain the *in vivo* shape of an organ without deformities, it can be placed in the anatomic position or submerged in a sodium chloride/water solution.

The polymerization time has been a very common problem. It can be prolonged or shortened as desired, by changing the catalyst concentration. This not only allows the modification in polymerization time, but also, the filling capacity, thus generating a better resolution, defined as further distribution of the polymer in the organ to smaller ducts, shown in Figure 5.

Table I describes each polymer, its components, different concentrations and uses, time required for previous preparation of the polymer or mixing time, hardening time, and recommended injection time. The last column gives a reference on the recommended duct diameter for each polymer for better results.

Corrosion. This process requires careful handling of substances by the personnel working with the specimen, because of the corrosive characteristics of the chemicals used. We recommend referring to the institutional safety manual and using all the necessary security elements for the manipulation of the chemical agents.

Many agents have been described for this process, among which is sodium hydroxide (NaOH), known as caustic soda. This has not only shown efficacy, but also flexibility

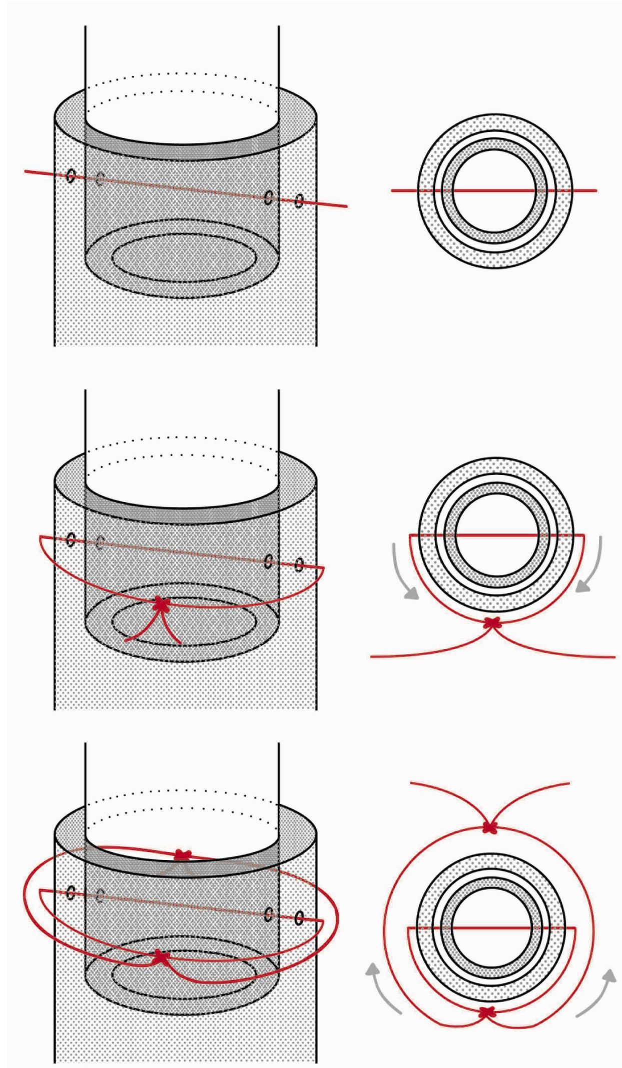

Fig. 3. Suggested suture pattern for cannullation. Transfixion suture ensures the tube stays firmly inserted in the duct and prevents leakeage due the pressure while being injected.

concerning corrosion times. The tissue hydrolysis can take up to 6 months at low temperatures (room temperature), depending on the submerged tissue and can be accelerated by heating the NaOH solution to a point in which complete hydrolysis can be achieved within a few hours. It is important to note that NaOH can perforate iron or aluminum containers, so the process must be done in a stainless-steel container if the reaction is going to be sped-up. If done at low temperature, ensure that the container material has no organic matrix, or this matrix will also be hydrolyzed. For the residue disposal after finishing, we recommend inactivating the strong bases (like NaOH) with weak acids like acetic acid (vinegar). The amount or vinegar needed depends on the initial NaOH concentration. The remaining reactive should not be disposed directly in the drain due to its corrosive and contaminant characteristics.

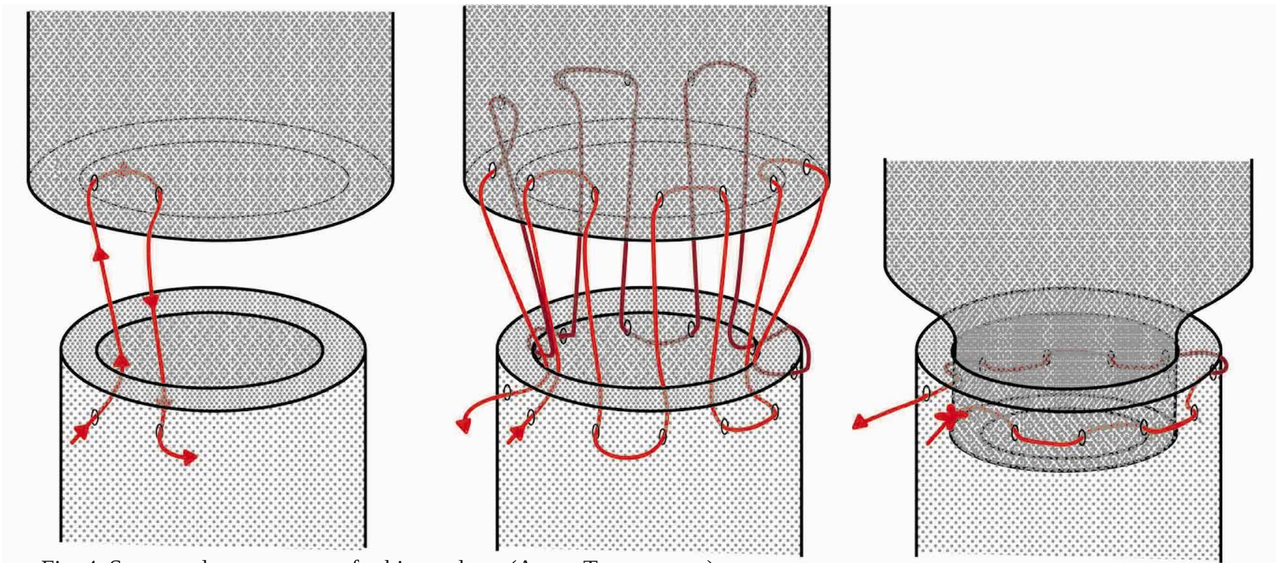

Fig. 4. Suggested suture pattern for bigger ducts (Aorta, Traquea, etc.).

Fig. 5. Different levels of resolution, achieved by obtaining a high-resolution specimen and fracturing delicate fragments to allow bigger structures to be uncovered. Left: Top: High resolution, shows alveolar capillary circulation and anastomosis. Middle: Balanced resolution, demonstrates correlation between alveolar and terminal bronchii circulation. Below: Early divisions of arteries and main veins, divided in different lobes. Right: Frontal view of the complete specimen.

RESULTS

Examples of the final products obtained after this process are provided below, a variety of specimens injected with different polymers show the characteristics of the final piece after both corrosion in low and elevated temperatures as shown in figures 6 and 7 respectively.

One finding when using high temperatures to speed up the processes is the deterioration of the fine details of the specimen. When using high concentrations of the strong base at low temperatures the corrosion did not take place, a concentration below 25% (weight/volume) was found to be most effective and is currently used in the laboratory. With this corrosion technique, the details are preserved, and a higher resolution can be attained.

The Figure 8 shows the correlation achieved by injecting both vasculature and airway, with later fracturing of the corroded specimen to expose arteries and veins, an effect equally observable in Figure 6 in which the injected ducts have similar resolution.

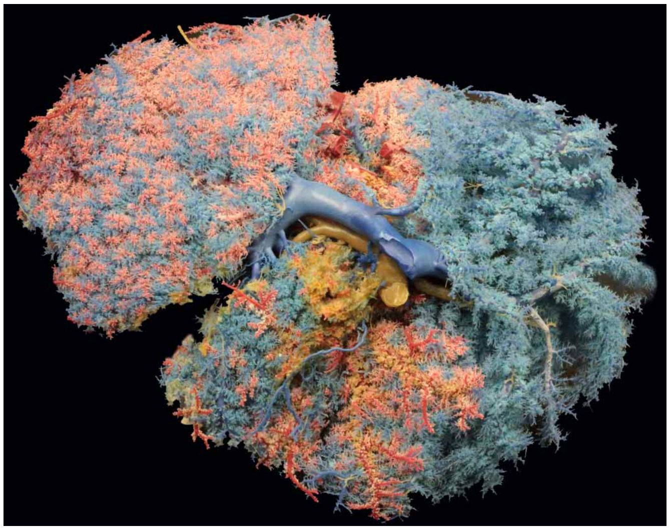

Fig. 6. Porcine liver inyected through the biliary duct (green), hepatic artery (red) and portal vein (blue), using acrylic, corroded with NaOH at low temperatures.

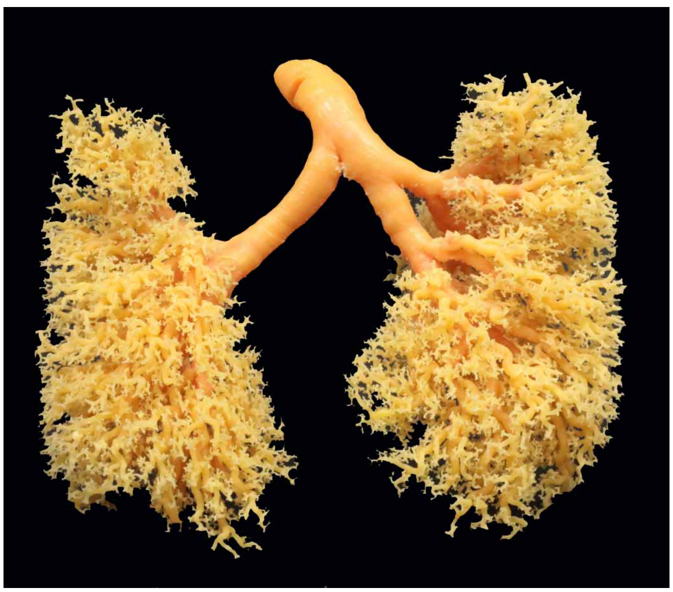

Fig. 7. Human lungs injected through the traquea with epoxy resin and corroded with NaOH at high temperatures.

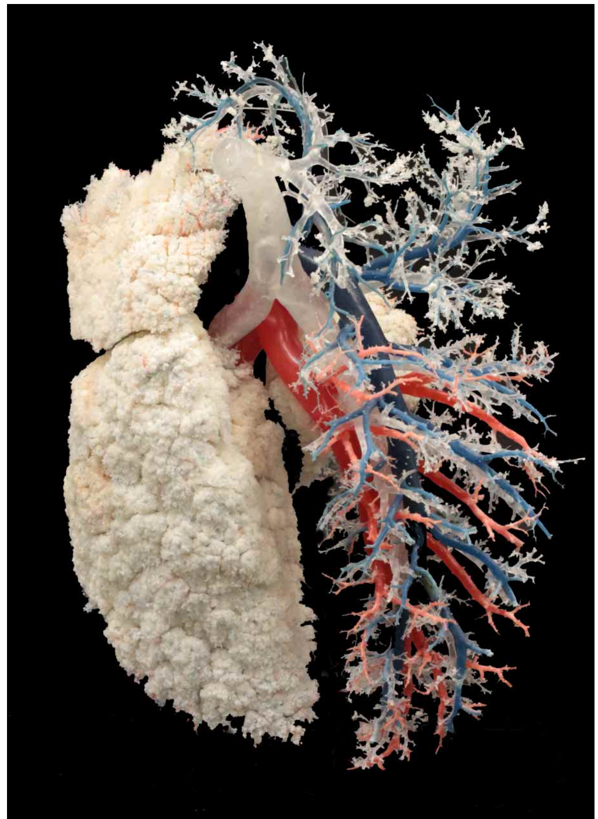

Fig. 8. Porcine lungs injected with acrylic at very low viscosity to achieve complete distal perfusion and high resolution of the airway, with different layers of resolution achieved by careful fracturing after the corrosion process.

DISCUSSION

This paper describes the corrosion casting technique to produce anatomical specimens of high quality and resolution. Authors have decided to use resolution as a term to describe how deep the polymer enters in the injected ducts, allowing the preservation and visualization of very small and almost microscopic structures.

The injection and corrosion technique permits the procurement of specimens with a very high academic and didactic value. This is so, because it correlates the tridimensional shape and the interior organization of the treated structures, a classic problem in textbooks. Injection with a very high-resolution technique must be managed very carefully given the frailty of the final products. Therefore, we recommend them to be kept in water on a definitive container to prevent any fracture caused by manipulation. When used for teaching purposes, these pieces, even though valuable have shown to be extremely fragile and must be treated carefully or they will lose their integrity under careless or inexperienced hands.

At the Universidad de los Andes School of Medicine the injection and corrosion technique was first introduced in 2006, and it has been considered by many students as a valuable tool for anatomy learning. The fact that students are included in the process of making pieces that will be used, not only by them, but also by generations to come, is thrilling and makes this technique very useful in teaching other aspects of organ applications, not only the ethical aspects in the treatment of specimens, but also in biosafety and other educational issues that are important components of today's medical formation. Often, the students regard biosafety as something for the protection of someone else and not their own. Manipulation of fresh specimens and the injection-corrosion process, teaches them about the physician's commitment to self-protection and care.

In terms of teaching and learning, there is not only an appropriation of knowledge related to the specimen, but also an increased opportunity of peer learning. It is customary practice at the Anatomy Laboratory for students to learn from those who have developed one specific project. This has fostered fellowship and teamwork, increasing the appropriation of new concepts, not only the students working on the piece, but those in the rest of the class. This generates in the students a better attitude towards the course.

CONCLUSION. The use of corrosion casting technique in the Anatomy Laboratory has shown, not only an increase in the number of students working on their own dissection projects, but that it also helps students in diverse matters with their anatomy teaching and learning process. It is not only useful for students using this technique, but it is also a source of didactic aids for their theoretical and practical sessions. The specimens obtained have provided a better understanding of the internal tridimensional structure of an organ and have facilitated the acquisition of new concepts in irrigation, blood draining, the hepatic portal system, the bronchial-vascular relation of the lungs, among others, both in theoretical and practical sessions. Furthermore, it has demonstrated excellent results in helping students understand the internal composition of an organ that is going to be dissected. This allows the students to make a mental map of the structure they are going to dissect and a tridimensional image of the organ and the vasculature that compose it, to have a better understanding of the organ before picking up a scalpel. This is beneficial because it decreases the dissection time and minimizes the damage done to dissectible specimens due to the lack of knowledge.

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RESUMEN: El proceso de aprendizaje y enseñanza en anatomía ha cambiado recientemente, el cadáver fue la primera herramienta que permitía al estudiante una aproximación practica al cuerpo humano, facilitando que este se sobrepusiera al conocimiento teórico adquirido en el aula, y desarrollara experticia aplicada. Hoy en día, técnicas como la inyección corrosión son conocidas por lograr mejores resultados con los estudiantes. Este trabajo presenta un método para aplicar esta técnica a un órgano, mediante el uso de diferentes polímeros. Las concentraciones adecuadas de acrílico, resina epoxica, resina poliéster, y silicona RTV (room temperature vulcanization) son descritas, así como el diámetro sugerido para el uso de cada polímero. Se presenta una variedad de especímenes obtenidos mediante esta técnica, así como sus cualidades y características. Al integrar a los estudiantes en el proceso de creación de los especímenes, estos demuestran mayor sentido de responsabilidad, dedicación y autoconciencia, generando mayor compromiso, y entusiasmo con la clase y el proceso de aprendizaje. Adicionalmente, los especímenes producidos por ellos, serán de gran utilidad para clases a futuro.

PALABRAS CLAVE: Inyección corrosión; Repleción; Corrosión; Educación en anatomía; Técnicas de preservación.

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Corresponding author:
Roberto Javier Rueda Esteban
Postal Address: Carrera 1 No. 18a -10
Sótano Y006, Laboratorio Anatomía
Bogotá - COLOMBIA

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E-mail: rj.rueda32@uniandes.edu.co