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A New Method to Make Vascular and Bronchial Casts of Voluminous Organs

D. Viggiano¹, S. Sangiorgi², M. Reguzzoni², A. Manelli², L. Marano¹, C. Dell'Orbo² and C. Passiatore¹

¹Department of Medicina Pubblica, Clinica e Preventiva (Human Anatomy) and Department of Experimental Medicine, Second University, via Arena, 5 – Loc. S. Benedetto, 81100 Caserta, Italy, ²Laboratory of Human Morphology, 'L. Cattaneo', University of Insubria, via Monte Generoso 71, Varese, Italy

Abstract

Vascular and bronchial endocasts represent a useful instrument to study the ramification pattern of these structures. Casts have been made from different materials, such as waxes in ancient times and, more recently, silicon-like compounds or resins (see e.g. Mercocox) to study the finest details. These techniques are valuable for small specimens, whereas they are inadequate for very large organs, where technical difficulties require the development of specific instrumentation. In this study we present a new simple injection technique, based on expanded polyurethane, which allows preparing vascular and bronchial trees for macroscopic and microscopic studies. The new injection technique is very easy to carry out, since the propulsion is provided by compressed air, and it does not require special instrumentation. To this aim, endocasts of the entire tracheal–bronchial tree and casts of vascular kidney from different animals were prepared. The specimens have a very low weight, show the finest ramifications, and are very stable and resistant to mechanical stress. To examine microscopically the details of the casts, specimens from the kidney cast were also analyzed by scanning electron microscopy, revealing good preservation of microcirculatory structures, functional sphincters and endothelial cell impressions. Therefore, the technique may be useful for macroscopic studies of large specimens, retaining sufficiently fine details.

Keywords: Corrosion cast, vasculature, bronchial tree, kidney, scanning electron microscopy, polyurethane.

Introduction

As early as the end of the XIV century, Leonardo da Vinci already knew of the use of injectable materials for the study

of vascular casts. In 1800 the most used injection material was a mixture of waxes, a minimum as coloring agent, and turpentine oil, as in Ruysch and Hirtl's wonderful models (see also Passiatore & Mezzogiorno, 1994). Over the last century a variety of materials have been proposed, such as celluloid (Schummer, 1935; Peterson, 2004), vinyl resins (Narat et al., 1935; Kazzaz & Khanklin, 1950; Stern et al., 1954; Tepperman & Pearlman, 1958), latex gum (Schlesinger, 1938; Batson, 1939; Gamble, 1939), acrylic resins (Tompsett, 1959; Schafe et al., 1973), polyester (Nerantzis et al., 1978; Northover et al., 1980), silicone (Yonas et al., 1982; Nettum, 1995), latex neoprene (Smith & Henry, 1945), nylon (Wagner & Pondexter, 1949; James, 1961; Smith, 1962; Fulton, 1965; Hodde & Nowell, 1980). Murakami (1971) emphasized the utility of methyl-metacrylate (Mercocox) semipolymerized to study the microcirculation using scanning electron microscopy (SEM) (Ditrich & Splechtna, 1990; Lametschwandtner et al., 1990; Schraufnagel & Ganesan, 2002). This method is also useful for studying the superficial properties of the cast and the imprints of cells on the resin (Hodde & Nowell, 1980; Konerding, 1991), though the method may present some artifacts (Aharinejad & Böck, 1993). Unfortunately, these endocasts are fragile and relatively heavy so that, particularly in the case of large organs, the casts may show distortions due to their own weight. Moreover, the technique is expensive, thus being better suited for the study of small specimens. Therefore, alternative materials are necessary when analyzing entire body districts. Latex and nylon can be injected into very thin vessels, but the final cast must be preserved in a fluid to prevent distortions and curls (Stern et al., 1954). Moreover, latex is not hard, requiring supporting casts (Wagner & Pondexter, 1949). Vinyl produces specimens sub-

jected to very intense coercion (Smith, 1962; Wolfe, 1956). Polyester has very good macroscopic characteristics, although the quality of surface at SEM is not known (Nerantzis et al., 1978). Acrylic resins can soak vessels walls (Tompsett, 1959). The study of voluminous organs is important to identify patterns of vascular effects, to study changes in the microcirculation in different regions, and to realize subsequent mathematical models of their ramification pattern. Ideally a method to study large specimens should be technically reproducible, the endocasts should retain their shape (Hanstede & Gerrits, 1982), and it should be economically advantageous. Therefore, the injection material (i) should be light, so that it does not collapse under its own weight, (ii) should not be too hard so that it does not crack when manipulated, (iii) should penetrate into very fine vessels without soaking the walls, and (iv) should retain enough information about the microscopic detail of the surface of the injected vessel.

To this aim, we developed a new technique based on expanding polyurethane propelled by compressed air, that results in very stable casts which, when analyzed by SEM, preserve good details of the microcirculation and cellular imprints.

Materials and methods

Injection material

The injection material is a commercial product (rapid foam, Saratoga Int. Sforza s.p.a., Milano, Italy, cat. 85208), already prepared for propulsion with compressed air. Polyurethanes are a class of polymers produced by two components: poly(ethylene glycol) and diisocyanate(bis-4,4'-isocyanophenylmethane). Diisocyanate has two cyanate groups and polyol alcohol groups. In the presence of diazobicyclo(2,2,2)octane or DABCO alcohol and cyanate residues it forms polymers with urethane bridges. In the presence of H₂O the reaction forms CO₂ that causes polymer expansion during solidification. Polymerization occurs at room temperature, giving rise to a stable compound. By mixing these two components, the mixture expands and the result is stiff expanded polyurethane, which fills the cavities of vessels, reproducing their shape. The mixture is a closed-cell foam with a compact surface that reproduces with high definition the surface of the cavity filled by the foam. The polymer has a high resistance to bumps and compression, dimensional stability and a low index of water absorption. Moreover, it is resistant to concentrated NaOH, thus allowing the maceration of the tissue. The foam in free expansion increases its volume by 43%. However, the foams obtained in a closed volume (such as after injection in vessels) are much more compact than those obtained in free expansion. The liquid mixture is expelled by propelled air.

It is quickly injectable and solidifies uniformly. The time of complete hardening is approximately 2 h, although after 20 min it acquires such consistency that it can be cut. This

substance is impermeable, unassailable from chemical agents, solvents and moulds. The temperature of use for an optimal result is within 18–22°C; the temperature of exercise from –40 to 100°C, the temperature of application from 5 to 35°C.

The injection material is economical, and can be transported easily and preserved for at least 1 year.

Organs

The organs derived from animals slaughtered 24 h before the procedure. They were not submitted to perfusion or other treatments before the injection. The following specimens have been examined: the broncho-vascular tree of (i) sheep (*Ovis aries aries*) and (ii) pig (*Sus scrofa domesticus*), injecting the bronchial tree, the pulmonary artery and the origin of the pulmonary veins at the same time; in the latter, the aorta was also injected to demonstrate its relation with the lungs; (iii) the arterial tree of cow (*Bos taurus*) kidney, injecting via the renal artery.

In order to examine the reproducibility of the technique, for each organ two separate casts from two animals were prepared.

Casts were photographed after complete maceration and observed macroscopically or under optical conventional microscopy to analyze details of the surface. Only kidneys underwent further analysis by scanning electron microscopy.

Method of injection

In order to get the vascular endocast, a cannula 0.4 cm in diameter was inserted into the main conduit of the isolated organ. Since the injecting material is hydrophobic and sticks to dry surfaces, it is useful to wet the organ surface and the workbench; it is also advisable to perfuse the organ with physiological solution before the injection (although in the present paper the organs were unperfused). A forceps was used to maintain the cannula inside the main vessel of the organ, to achieve a uniform injection pressure. The cannula was then connected to the compressed air device containing polyurethane. This was injected until small vessels on the organ surface were visible (the injection material has a white color which contrasts with the darker aspect of the organs). During injection the polyurethane cylinder was maintained vertically up-side-down to obtain uniform propulsion of the polyurethane.

Since the injection material is not a liquid, but a foam, and the propulsion is by compressed air, the injection pressure must change over time to ensure correct injection. In fact, at the end of the cannula there is a drop of pressure up to 0.1 atm if the end is open. However, when the cannula is completely closed, the pressure will increase until equilibrium with the pressure inside the air-compressed cylinder (1 atm) is achieved. When the cannula is attached to a high resistance pipeline, such as the vessels of an organ, the pressure will increase until the foam can enter into all the pipes. After-

wards, the resistance of the same foam inside the vessels would give rise to an increase of the inner pressure up to equilibrium with the pressure inside the compressed-air cylinder. However, this never happens if the injection is stopped when the foam enters into the small capillaries (which are evident on the surface of the organ as the foam is white, contrasting with the color of the organ). Therefore, the pressure of the injection can be operationally defined as that necessary to allow the foam to enter into capillaries. If the pressure is, instead, maintained constant, the flux varies depending on the state of contraction of the vessels, especially for capillaries.

At the end of the injection the cannula was left for 5 min *in situ*, to allow complete polymerization. Moreover, the organ was left undisturbed for further 20 min, to avoid any distortion of the vessels. The organ was then immersed in 10% NaOH at room temperature, renewing the liquid every day. After complete maceration the cast was air-dried.

SEM preparation

Three polyurethane casts of cow kidney, already dried as above, were cut into little pieces under a dissecting stereomicroscope. The glomeruli were identified under the stereomicroscope, and pieces of interlobular arteries with their afferent arteries, glomeruli and efferent arteries, were isolated using fine scissors. The specimens were mounted on aluminum stubs using an adhesive film. In some cases it was necessary to use metal bridges to make the specimens conductive. The mounted stubs were then coated with 10 nm of gold in an Emitech K250 sputter-coater and observed using a Philips XL-30 FEG scanning electron microscope operating at 10 kV.

Results

Immediately after propulsion, the polymer solidification begins. It may contain small bubbles of CO₂, which can be observed under both the optical and scanning electron microscope.

The endocast of the lung showed the ramification of the bronchial tree in both sheep and pig (Fig. 1). In the latter, the regionalization of the lung was also visible, with typical segmental organization. The vessels and alveoli were also well represented by the endocast. The number of peripheral branches injected was so high that the peripheral vessels had to be removed in order to observe the structure underneath (Fig. 1A). The ramification pattern of airways and pulmonary vessels was also evident, with typical branching of terminal ramifications. Asymmetrical dichotomy divisions were preponderant in the conduction tree, as previously described (Maina & van Gils, 2001). In spite of the large dimensions of the entire bronchial and pulmonary vascular tree of *Sus scrofa*, the resulting cast was very light and stable.

The cow kidney cast also showed the peculiar ramifica-

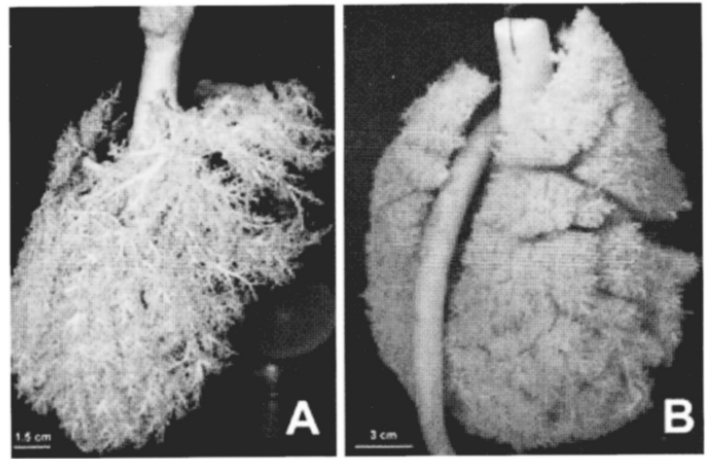


Fig. 1. Posterior aspect of bronco-vascular tree of *Ovis aries* (A) and *Sus scrofa* (B), obtained by injection of pulmonary arteries and trachea. In the preparation on the right the descending aorta is also visible (the intercostal arteries have been cut away). The regionalization of the lung is well visible in the preparation from *Sus scrofa*.

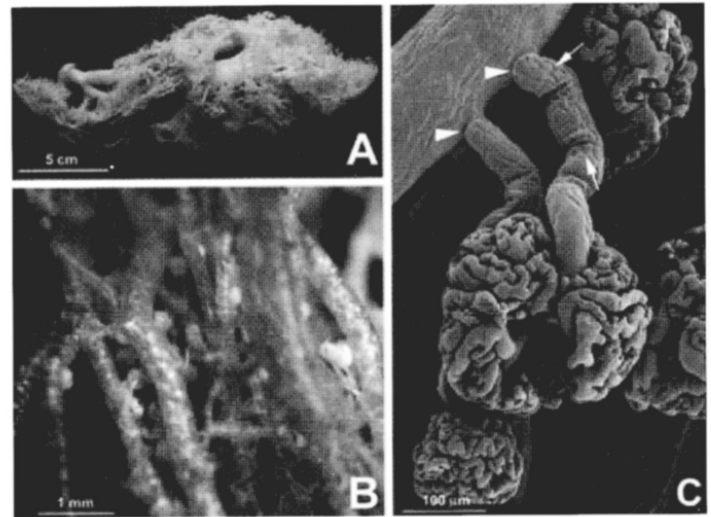


Fig. 2. Vascular tree of *Bos taurus* kidney injected with polyurethane. (A) Macroscopic aspect. (B) Low power magnification; note the glomeruli. (C) Scanning electron microscopy. Glomeruli, functional sphincters (arrowheads) and endothelial cell impressions (arrows) are visible.

tion pattern of arterial vessels (Fig. 2). In fact, most of this vascular tree was also injected with the polyurethane (Fig. 2). The cast respected the typical branching pattern. Low power magnifications (Fig. 2B) showed that the polyurethane entered into the glomeruli. Using the scanning electron microscope we could clearly follow the fine vascular architecture of the kidney starting from the arcuate, interlobular and interlobar arteries through to the capsular capillaries. It was possible to observe, using optical microscopy and SEM, a typical annular caliber reduction in many vessels, which can be interpreted as functional sphincters. Inspection by scanning electron microscopy revealed that the polyurethane filled the thinnest sections reaching the glomerular vessels. However, most of the glomeruli miss the efferent artery. No

extravasations were observed, nor were artifacts due to blood clots or aggregated red blood cells. In fact, as observed by SEM, injecting liquid did not exceed the artery wall. Using SEM, the compound surface showed the prints of the endothelial cell nuclei. In fact, over the cast of either big or thin vessels it was possible to observe the ovoid impressions due to the nucleus of endothelial cells and the ring-like depressions due to the sphincter systems that lie around the vessels. However, the presence of air bubbles in the compound (see Fig. 2B) greatly limits the use of this material for ultrastructural studies, where alternative techniques (e.g. Mercox) should be preferred.

Discussion

In this study we applied this technique to sheep and pig lungs, and also to cow kidney. The method proposed is easy, reproducible and economically advantageous, and allows realization of casts of voluminous organs. It is advisable principally for the macroscopic study of ramification patterns, because, in contrast to other techniques, it produces very light and stable preparations. The easiness of the technique resides in the use of propelled air as the propulsive medium. The same propulsive medium was also previously proposed for Silastic 734 RTV, injected with compressed air, which gave a good description of microcirculation details. Blood vessels were also studied utilizing Araldite CY223 as injecting liquid (Van Der Zwan & Hillen, 1990), which has a viscosity similar to that of blood, or Murakami's method (1971). However, in voluminous organs, like the lungs of pig, these methods are expensive and the resulting cast is so heavy that it is easily damaged or may undergo to distortions (Ohta et al., 1990). For these reasons, alternative materials have been proposed, such as silicone rubber (Nettum, 1995). The injecting technique presented here allows realizing dimensionally stable casts, thus allowing their transportation without damage. Moreover, the polyurethane foam enters very fine vessels, and adequately reproduces the morphological details of the surface on a microscopic scale. For its mechanical properties and technical easiness, with good results on microscopic observation, this tool is potentially useful in the study of anatomic variations of voluminous organs, in physiological or pathological conditions, and subsequent global research of microcirculation. Therefore, it represents an alternative to corrosion techniques with methyl-methacrylate, which is more indicated for microvascularization studies on small specimens. It is possible that the expansion of the foam causes an enlargement of the caliber of small vessels. However, on SEM observation, the morphology is comparable with that described by other authors (Ditrich & Splechtina, 1990).

Therefore, although Murakami's technique is a very good procedure for morphological studies using SEM (Gorczyca et al., 1994), expanded polyurethane can be used for macroscopic investigations, to have a global vision of the vascular tree while keeping acceptable microscopic details.

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