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COMBINED BRONCHOALVEOLAR-VASCULAR CASTING OF THE CANINE LUNG

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Abstract

Canine bronchoalveolar and vascular corrosion casts were prepared using unfixed tissue and Dow-Corning Room Temperature Vulcanizing Silastic® 734. The casts were observed using stereo light microscopy and scanning electron microscopy. The casts show the relationship between the vasculature and airway and demonstrate intricate microanatomical details. Also, microvasculature filling was enhanced using unfixed tissue as compared to my previously described technique using fixed tissue. Prewashing the microvasculature with cold phosphate buffered saline appeared to facilitate microvascular filling with silicone rubber. The described method is useful for rapidly making durable models for studying the normal respiratory airway and microvasculature. It should be useful in future studies of diseased pulmonary tissue as well as other normal and diseased tissues where microanatomical relationships between microvasculature and adjacent luminal structures are relevant.

Key Words: Silicone, lung, cast, bronchopulmonary, pulmonary arteries, pulmonary veins, alveoli, canine.

Introduction

Casting has been an exceptional instrument used to investigate the microanatomical structure of the pulmonary vasculature and airway. Both Schraufnagel (1987), in his review of microvascular corrosion casting of the lung, and the updated vascular corrosion casting review of Lametschwandtner *et al.* (1990), have documented the state of the microvascular casting field. Schraufnagel and Schmid (1988a,b) concluded that rinsing the lung microvasculature prior to casting improved the frequency of obtaining completely filled casts. Schraufnagel (1989) used casting to study both the bronchial and pulmonary microvasculature.

Many others have used a variety of lung airway and/or vascular corrosion casting techniques: Narat *et al.* (1936), Liebow *et al.* (1947), Rahn and Ross (1957), Tucker and Krementz (1957), McLaughlin *et al.* (1961), Frank and Yoder (1966), Eisman (1970), Nowell *et al.* (1972), Phaelen *et al.* (1973), Haefeli-Bleuer and Weibel (1988), and Wang and Kraman (1988).

Nettum (1993) used formalin-fixed canine lung to cast the airway with Dow-Corning's flowable, white, Room Temperature Vulcanizing Silastic® 734 (Dow-Corning, Midland, MI). Subsequently, the procedure was modified and both canine airway and pulmonary artery and vein were cast using stored, formalin-fixed canine lung (Nettum, 1995). In this study, fresh unfixed lungs were used to cast both the airway and pulmonary vasculature. Improved filling of the microvasculature is observed in these casts derived from unfixed lung.

Methods

Dog heart-lung blocks were immediately extracted following cardiovascular research activities. The apex of the heart was excised. The heart was grasped with one hand and with the opposite hand, a plastic nozzle was inserted into the pulmonary artery just past the cusps and in front of the bifurcation. The heart-lung block was then placed in a rectangular, 52 cm long, 43 cm wide and 17 cm deep, aluminum pot containing 38

liters of tap water. The myocardium around the nozzle was then squeezed creating a tight seal. Both pulmonary artery and vein were then washed with 4°C phosphate buffered saline from a 25 liter container at a height of 2.35 meters. The heart-lung block remained submerged until the vascular washing was completed. During the vascular washing, the heart-lung block was submerged and then agitated up and down displaying a constant plume of blood exiting the pulmonary veins and into the water bath. The lung rapidly became cold, white, and the blood plume stopped.

The specimen did not become grossly edematous when the vasculature was rinsed or when the airway and vasculature were filled with Silastic 734 (Dow-Corning or Accumetric Inc., Elizabethtown, KY). A tissue section showed that alveoli were intact following vascular rinsing with cold saline. The heart-lung block was then rapidly transferred to another rectangular, 32 cm long, 25 cm wide and 11 cm deep, 6 liter clean tap water filled, plastic container. It remained in that container until a plastic cartridge nozzle (#TS618, Accumetric Inc.) with attached 1/10 gallon (0.378 l) cartridge gun assembly containing a 305 ml cartridge of blue pigmented Silastic 734 was inserted past the mitral valve in front of the pulmonary vein ostia. The lumen at the injecting end of the nozzle was 3 mm x 3 mm. The cartridge gun assembly was connected to an air compressor regulated to a maximum of 4.0 kg/cm^2. The heart was grasped in one hand and with the other hand the myocardium was squeezed creating a tight seal around a 25 mm wide and 2 mm high duct tape strip, the distal edge located 34 mm from the injecting nozzle's distal end. The specimen was submerged in the water bath and was agitated up and down. The blue Silastic 734 was rapidly and steadily injected into the pulmonary veins. The filling pressure dropped to 2.5 $\pm$ 0.25 kg/cm^2. The veins were considered full when blue Silastic oozed out through the tight fingered seal; the pleural surface became granular; the feathered peripheral edges became blue; and the lung became firmer. The heart was then raised above the water line, the nozzle was removed from the left atrium, an appropriately sized, tight-fitting, natural non-holed cork was rapidly inserted into the mitral valve with minimal loss of blue Silastic 734 and pinned in place until polymerization was completed. Immediately, the same process was done using red pigmented Silastic 734 in the pulmonary artery. The feathered peripheral edges became red and the lung became noticeably firmer. Injecting the vasculature with colored red and blue Silastic 734, produced a multicolored granular visceral pleural surface.

The lung presented as a multicolored, blue and red specimen without color extravasating into the tissue. Immediately, the same submersion-agitation technique was used, injecting the contents of a 305 ml white (opaque) Silastic 734 RTV (viscosity, 440 poises with limits between 200-500; specific gravity at 25°C, 1.04; and 3 mm deep surface cure in 24 hours) canister into an unwashed tracheobronchial tree. To accentuate the vascular cast and de-emphasize the airway, white Silastic 734 was not injected to produce the pleural granularity indicative of alveolar filling. The airway filling process was stopped when white Silastic 734 was just visible through the transparent visceral pleura. The proximal end of the mainstem bronchus was clamped with the lightest and the smallest fitting ACCO Binder Clip (ACCO USA, Wheeling, IL). The heart-lung block was then floated or submerged for 72 to 96 hours in 10% neutral buffered formalin to maintain anatomical shape and assure cast polymerization. Following polymerization and fixation, the corks were removed from the pulmonary artery and vein. The heart-lung block was then placed in a previously heated, two molar potassium hydroxide solution (40°C) and constantly agitated for five days using a shaker bath. During the week, the potassium hydroxide was replaced five times. A multicolored silicone rubber cast was recovered and washed in 50% ethyl alcohol; dehydrated in 100% ethyl alcohol; submerged once and agitated in 1,200 Prime Coat (Dow-Corning) in 100% ethyl alcohol (5% solution); immediately submerged once; agitated and washed in 100% ethyl alcohol; and then air dried.

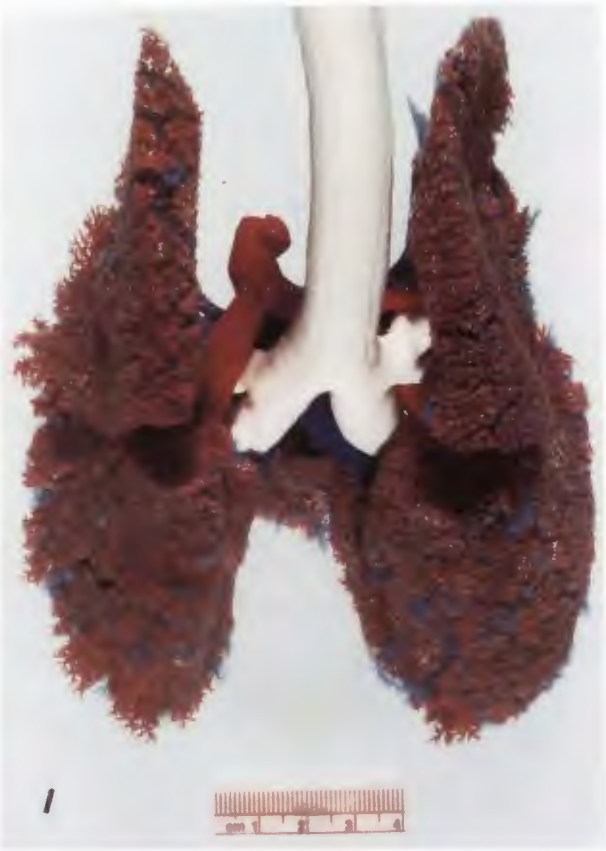
The samples for scanning electron microscopy were mounted on aluminum stubs with double sided tape (3M, St. Paul, MN). Sputter coatings using the Hummer I (Technics, Alexandria, VA) were approximately 20 nm in thickness using gold-palladium as the coating metal. The coatings were done in an argon atmosphere of 120 millitorr. The accelerating voltage was 15 kV.

Figure 1. A combined bronchoalveolar vascular corrosion cast. The vasculature's red and blue complex networking is contrasted with a prominent white mainstem bronchus.

Figure 2. A corrosion cast of two pulmonary lobes. Note the limited airway (white) filling and prominent red and blue vascular networking. Bar = 4 mm.

Figure 3. A corrosion cast illustrating a major pulmonary venous trunk (blue). The trunk is partially surrounded by a pulmonary artery (red) and vein (blue) microvasculature. Bar = 3 mm.

Figure 4. A combined bronchoalveolar-vascular cast showing a prominent pulmonary vein (blue), the pulmonary artery (red) and airway (white). Bar = 1 mm.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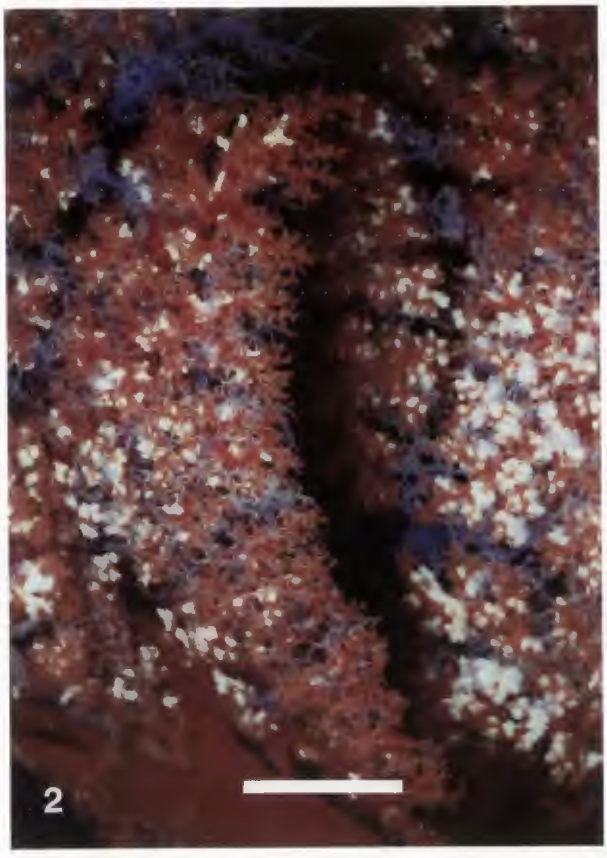
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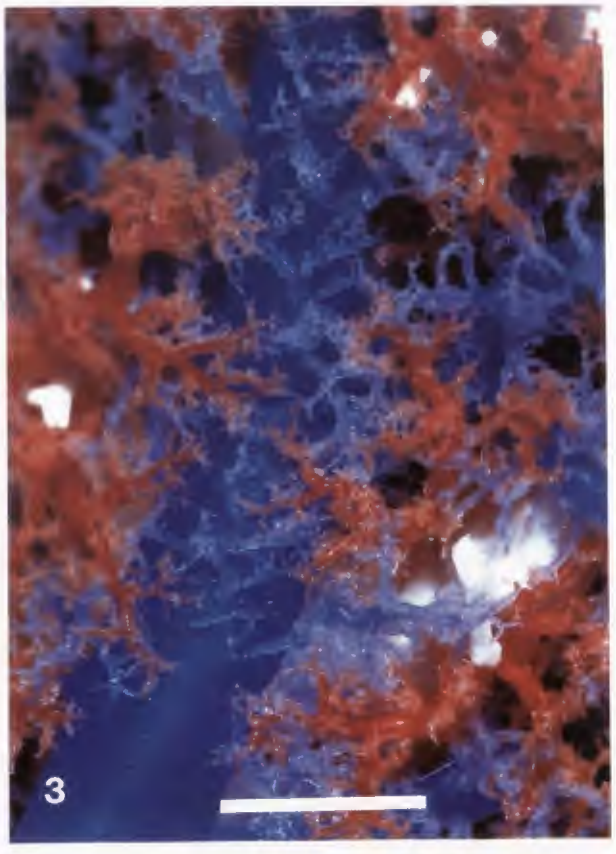
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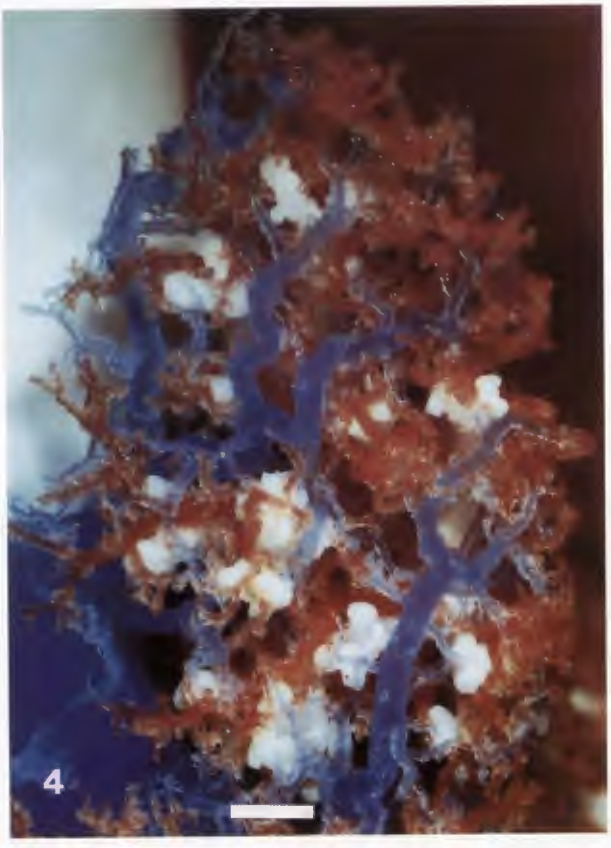
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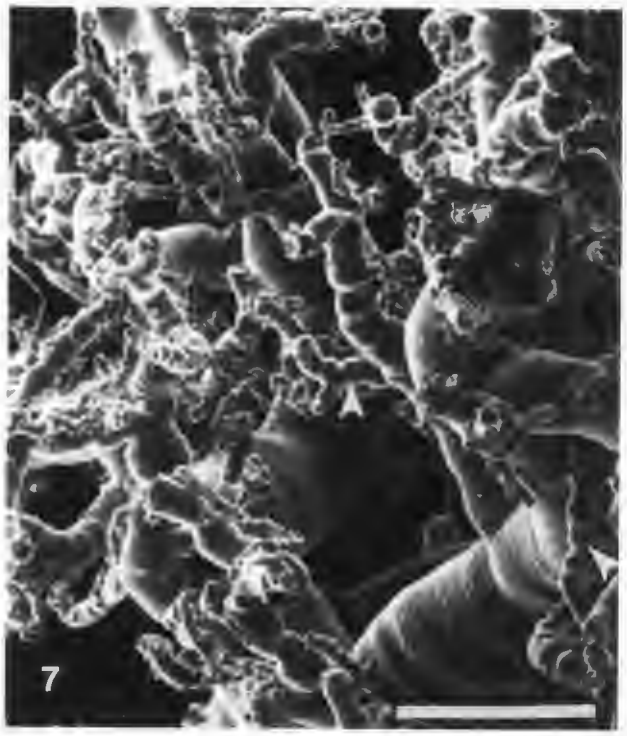
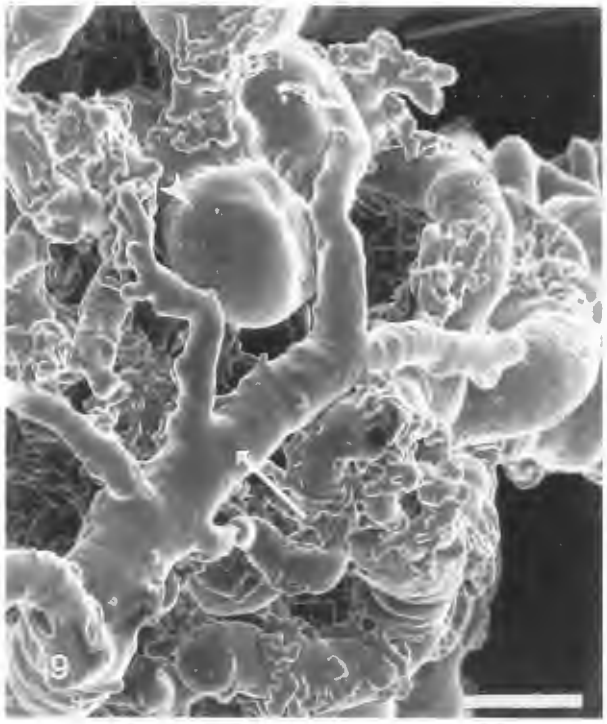
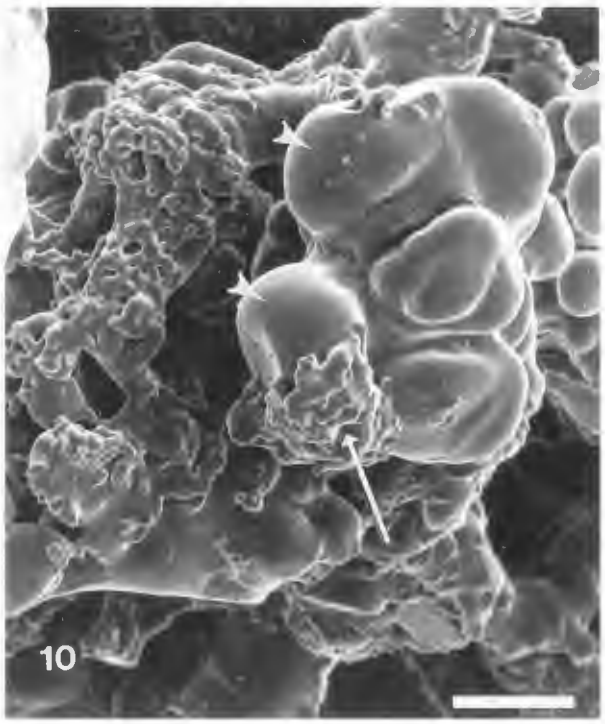
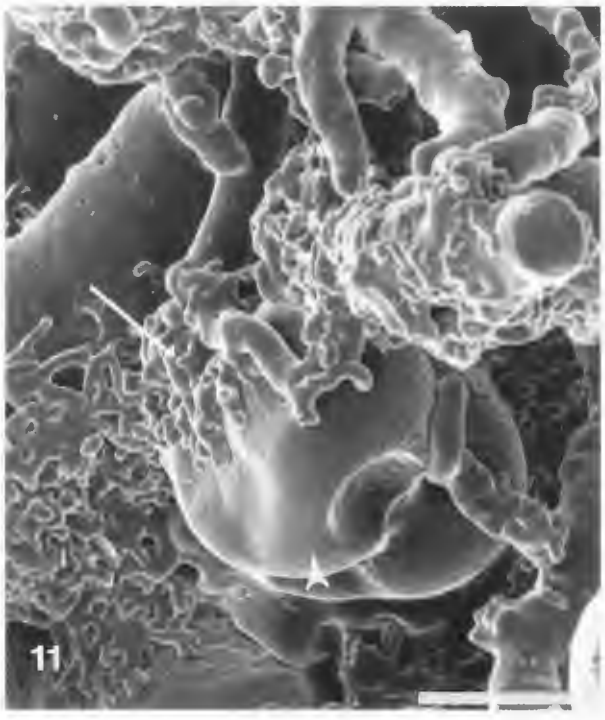


Results

Figure 1 shows the complex multi-colored networking or cob-webbing created by the pulmonary artery, vein and distal structures. As shown, the white airway was intentionally under filled, with the mainstem bronchus and its major branches providing the structural foundation and emphasizing the prominent vasculature. Figures 2, 3, and 4 are closer macroscopic views of Figure 1. Figure 2 displays two lobes with red and blue vascular networking with limited airway filling. Figure 3 depicts a pulmonary artery (red) and pulmonary vein







Figures 5-11. Scanning electron micrographs (**Figures 5-8 on the facing page**) of: **Figure 5.** A large blue pigmented pulmonary vein cast extracted from Figure 1; concentric bands are seen. **Figure 6.** The pulmonary vein at high magnification; smaller microvasculature has apparent annular contractions, focal bulging (arrows) and limited surface pitting. **Figure 7.** A red pigmented pulmonary artery cast extracted from Figure 1; note the increased number of surface pits and the distal microvasculature with corkscrew appearance (arrowheads). **Figure 8.** The pulmonary artery at high magnification; the distal microvasculature has a corkscrew appearance (arrow) and the same artifactual depressions on the cut surface are also present on the surface (arrowhead). **Figure 9 (above).** A combined bronchoalveolar-vascular cast; a prominent pulmonary venous structure (arrow), multiple alveoli (arrowhead), pulmonary artery microvasculature segments and capillaries structures are shown. **Figure 10 (above, right).** A combined bronchoalveolar-vascular cast; the capillary structures (arrow) partially surrounds alveoli (arrowheads). **Figure 11 (at right).** A bronchoalveolar-vascular cast at high magnification; the capillary structures (arrow) partially surrounds alveoli (arrowhead). **Bars = 100 μm (Figs. 5, 6, 8, 9, 10 and 11) and 500 mm (Fig. 7).**

(blue) microvasculature as it surrounds a large pulmonary venous (blue) trunk. In the background, the airway (white) is barely visible. In Figure 4, the blue pulmonary vein is prominent, forming a microvascular network adjacent to the red arterial vasculature and white alveoli. Figures 5 and 6 are scanning electron micrographs of the same corrosion cast. Depicted are separated and small pulmonary venous structures derived from the cast in Figure 1. Constricting bands or annular contractions are seen and appear similar to those shown in rat pulmonary veins by Schraufnagel (1987), Schrauf-

nagel and Schmid (1988a,b), Schraufnagel and Patel (1990) and Aharinejad *et al.* (1991, 1992). Also, apparently present in the wall of the vessels are focal bulges. Figures 7 and 8 are scanning electron micrographs of the same corrosion cast. Depicted are pulmonary arterial structures derived from the cast in Figure 1. Multiple small surface pits are seen and the distal microvasculature has a corkscrew appearance. Figures 9, 10 and 11 are scanning electron micrographs of a combined bronchoalveolar corrosion cast. A smooth capillary network appears to converge on alveoli (Figures 10 and 11). Also seen within the pulmonary venous microvasculature are a minimal number of surface pits while the pulmonary arterial microvasculature shows readily apparent surface pits. This pitting appears to be a property of the red silicone rubber rather than a property of the arterial vasculature.

Discussion

This study reports a method used to form resilient tracheobronchial-vascular casts from unfixed lung. These casts can be rigorously handled for gross demonstration, and the morphology can be studied in detail using stereo light microscopy or scanning electron microscopy. Injecting the pigmented Silastic 734 RTV into unfixed vasculature allowed for improved filling of distal vasculature. Using room temperature tap water to float, submerge and agitate the heart-lung blocks helped avoid mechanical obstructions (luminal or tubal kinking) during the filling procedure. The water bath also helped make the lungs weightless and easier to maneuver when observing vascular and airway filling during the casting process. Using 4°C phosphate buffered saline elevated at 2.35 meters, blood from the pulmonary artery was rapidly flushed through the capillary network and out the pulmonary veins. This cold washing step immediately chilled the lungs, appeared to slow autolysis, kept the microvasculature intact, and improved microvascular filling. Blue Silastic 734 RTV was first injected retrograde into intact pulmonary veins and then red Silastic 734 was injected following the physiologic flow of blood into intact pulmonary arteries without rupture. The pleural surface exhibited a red and blue granularity. After microvascular casting, the airway was under filled with white, opaque Silastic 734 creating no signs of white granularity on the visceral pleura. The airway provided contrast and accentuated the previously cast red arteries and blue veins.

In contrast to previous work using canine formalin-fixed lungs, improved vascular casts were made by washing unfixed lung vasculature with cold saline just prior to injection with Silastic 734. During Silastic 734 injection, the unfixed, rubbery myocardium was squeezed against duct tape wrapped around the injection nozzle. This created a tighter seal than previously published (Nettum, 1995), using formalin-fixed myocardium that had a holed cork inserted into the injection site prior to formalin-fixation. Also, unfixed lung tubal structures and lumens were more elastic than fixed lung, expanded extensively during casting, and allowed more time for clamping and cork plugging before silicone rubber escaped out the injection site. Once Silastic 734 was injected into both vasculature and airway, the specimen was placed in formalin. The unfixed vasculature then contracted around the polymerizing cast, enhancing imprints.

Scanning electron microscopy is readily used to study casts made from Silastic 734 RTV. For example, in the pulmonary artery or arteriole cast, small holes or pits are much more numerous than in the pulmonary vein or venule cast. The artifactual pits or holes help identify artery from vein. The holes appear very minimal at the capillary level. If these artifacts are created using red Silastic 734, it suggests capillary level perfusion from the venous side. Possibly, different metals, and/or soluble or insoluble powders, were used to color the red and blue pigmented paste that is blended into clear Silastic 734. The ingredients in the different colored pastes may account for the defects seen on the arterial cast. The red and blue pigmented pastes used to color Silastic 734 are proprietary.

Both artery and vein casts appear to demonstrate narrow, circular constrictions. Circular venous cast constrictions have been well documented in rat lung by Schraufnagel and Patel (1990) and Aharinejad *et al.* (1991, 1992). Therefore, this technique may offer the opportunity to further study this type of cast configuration in the canine venous and arterial system.

The methods described provide an efficient and convenient way to cast vasculatures, microvasculatures or airways, giving a permanent, three-dimensional structure that reveals microanatomical relationships with adjacent spaces. This injection technique using unfixed versus formalin-fixed tissue has several advantages including better microvascular filling and the opportunity to easily vary airway filling to accommodate microvascular, airway and microanatomical studies. Those studying normal and diseased tissues should find this technique generally useful.

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Discussion with Reviewers

D.L. Luchtel: Where does the saline in the vasculature go during casting of the vasculature from the arterial side after the venous side has been cast?
Author: After the venous side has been cast and when casting the arterial side, it was not studied where the saline goes. Grossly, the lungs are not edematous. This vascular casting technique has failed to completely cast the alveolar capillary bed. It may be too strong a statement to say that perfusion is incomplete secondary to back pressure caused by residual saline left in the pulmonary artery following retrograde Silastic 734 injection into the pulmonary vein. Incomplete filling may be caused by the rapid polymerization of Silastic 734 or secondary to its high viscosity.

D.L. Luchtel: If the lungs are not collapsed prior to airway casting, how well can the airway tree be filled with casting material?
Author: The airway is easily cast and alveolar dimensions correspond to what is in the literature.

D.L. Luchtel: Was the suggestion about the source of "pitting" ever tested, i.e., that the "pitting" of the arterial cast was a property of red silicone rubber rather than a property of the arterial vasculature; for example, by injecting the red silicone into the venous vasculature?
Author: The pitting on the arterial cast looked artifactual because it was on both the surface and cut surface. I have never tested the red silicone rubber by injecting it into a vein.

D.E. Schraufnagel: The casting material most often

used to cast vasculature is methyl methacrylate. How would you compare Silastic 734 to methyl methacrylate? Under which circumstances would your recommend each material?

Author: Silastic 734 casts are rubbery, flexible, not brittle, extremely durable, handled rigorously by students. Once fully polymerized, the casts maintain the same size, shape, color and consistency. The Silastic 734 cast allows students to easily compare the broad, gross relationships between air spaces and vasculature without fear of breakage or permanent damage. Silastic 734 comes ready to use requiring no mixing like methyl methacrylate. Electron micrographs of a Silastic 734 cast will give reasonably fine feature detail, but will not provide the very fine surface detail or the alveolar capillary bed filling that the more fragile methyl methacrylate will give for scanning electron microscopy.

S. Aharinejad: The rinsing of lung and heart was performed with saline. Does not saline (alone) cause perivascular edema?

Author: Perivascular edema was not studied by light or scanning electron microscopy.

S. Aharinejad: The container filled with saline was 2.35 m above the heart level. Is the pressure caused thereby not too high?

Author: Cold, 4°C saline at 2.35 m above the heart rinses lung vasculature extremely fast without rupture provided the heart-lung block is immediately extracted from the animal and rinsed without delay, slowing autolysis.

S. Aharinejad: Where did you purchase Silastic 734?

Author: Silastic 734 can only be purchased from a licensed Dow-Corning distributor. To locate the nearest distributor, call Dow-Corning, Midland, Michigan (in USA at 1-800-248-2481). Dow-Corning only sells merchandise though its own distributors. Adding color to Silastic 734 is done by a repackager who works with a distributor.

Tim Love, manager at Accumetric, Inc., Elizabethtown, KY 42701, had indicated (personal communication) that he will mix red and blue pigmented paste into Silastic 734. Recently, he has successfully mixed red and blue dye (not pigment) into clear Silastic 734 for me. The resulting vascular casts are excellent. His phone number is 502-769-3385. Accumetric also sells clear or white Silastic 734. Initially, clear Silastic 734 was colored with a red and blue pigmented paste through Chuck Killian, Crown Distributing, Bridgeton, MO 63044. His phone number is 316-682-1221.

S. Aharinejad: Is Silastic 734 (fully) alcohol resistant?

Author: Dow Corning does not market Silastic 734 as being solvent resistant. In the methods, ethanol has two uses: To first rapidly wash and dehydrate the cast and then wash off the 1200 Prime Coat in 100% ethanol mixture (a 5% solution). If this mixture is not immediately washed off in 100% ethanol, the cast will become white and brittle. Tackiness in the cast may be created by heating the potassium hydroxide during corrosion and changing the polymer chemistry of Silastic 734. The 1200 Prime coat solution will eliminate that tackiness. If the tackiness is still present, it must be taken back into the same or preferably a new 1200 Prime Coat solution again. Ethanol may have some influence on the red and blue pigment in the Silastic 734 cast. Bumping the cast against the sides of the container while washing and dehydrating in ethanol or extensive washing in ethanol may wash some color from distal, thin portions of the vascular cast.