

REVIEW ARTICLE

Casting Materials and their Application in Research and Teaching

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Abstract: From a biological point of view, casting refers to filling of anatomical and/or pathological spaces with extraneous material that reproduces a three-dimensional replica of the space. Casting may be accompanied by additional procedures such as corrosion, in which the soft tissue is digested out, leaving a clean cast, or the material may be mixed with radiopaque substances to allow x-ray photography or micro computed topography (μ CT) scanning. Alternatively, clearing of the surrounding soft tissue increases transparency and allows visualization of the casted cavities. Combination of casting with tissue fixation allows anatomical dissection and didactic surgical procedures on the tissue. Casting materials fall into three categories namely, aqueous substances (India ink, Prussian blue ink), pliable materials (gelatins, latex, and silicone rubber), or hard materials (methyl methacrylates, polyurethanes, polyesters, and epoxy resins). Casting has proved invaluable in both teaching and research and many phenomenal biological processes have been discovered through casting. The choice of a particular material depends inter alia on the targeted use and the intended subsequent investigative procedures, such as dissection, microscopy, or μ CT. The casting material needs to be pliable where anatomical and surgical manipulations are intended, and capillary-passable for ultrastructural investigations.

Key words: casting material, corrosion, microscopy, teaching, research

INTRODUCTION

In as far as biological specimens are concerned; casting means the process of filling natural or pathological hollow spaces of a specimen with an extraneous material. In the precorrosion casting era, both air and liquids were used (Aharinejad & Lametschwandner, 1992), but currently casting mainly utilizes solidifying or polymerizing materials. In the context of corrosion casting, corrosion refers to removal of the tissue surrounding the casted hollow space, so that the solidified cast depicts the three-dimensional (3D) architecture of the hollow structure, and thus the terminology corrosion casting (Tompsett, 1970; Hodde & Nowell, 1980). Casting in the concept of biological specimens should be clearly differentiated from casting of archeological zoolites and artifacts in which a replica is created from plaster, resins, or fiber glass (Goodwin & Chaney, 1994) or from casting as may be applied clinically in management of fractures (Boyd et al., 2009a, 2009b). Although the materials used may be the same, casts in the former case are reproductions of the object, made by pouring a liquid material, such as plaster or a resin into a mold, which has been made from the surface of the specimen. In dentistry, casting refers to the process by which crowns, inlays, and other metallic restorations are produced and may employ metals such as gold or titanium (Geurtsen, 2002).

BRIEF HISTORY

Since the description of arteries by Herophilus of Alexandria (325–255 BC), who performed the first ever human cadaver dissections (Wiltse & Pait, 1998), the need to understand the body vasculature and indeed hollow organs has heightened. Galen (circa 129–200 AD) established the basis of modern anatomy by compiling much of the knowledge obtained by performing dissections on animals and collections of drawings, based mostly on dog anatomy, which became *the anatomy textbook* for 1,500 years. The use of casts to visualize and describe morphological features of biological specimens is an old technique dating back to the 14th century when Alessandra Giliani, a young prosector, used colored liquids to view blood vessels (Aharinejad & Lametschwandner, 1992). The technique of wax modeling was already known to the Greeks and Romans but the first documented evidence in teaching anatomy was by Alessandra Giliani in Bologna, where she was a master in wax injection techniques. About a century later, Andrea del Verrocchio (1435–1488) was the first to make casts of the living body as models for schools and this apparently stimulated his pupil Leonardo de Vinci (1452–1519) to make wax casts of the ventricles of the brain (Cooke, 2010). In the subsequent years, investigators continued to use space filling substances including water, air, milk, ale, saffron, and ink. Swammerdam (1637–1680) injected colored wax in arteries and veins and other materials including starch, plaster of Paris, glue, glazier's putty, asphalt, gum Arabic (acacia gum), sodium silicate, sesame oil, shellac,

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thymol, and mercury (Wiltse & Pait, 1998). These investigators used casting without corrosion and this period is referred to as the precorrosion casting era (Aharinejad & Lametschwandtner, 1992).

The corrosion casting era started much later when Bidloo (1685) used Rose's metal (alloy of bismuth, lead, and tin) as an injectable and boiled the specimen to remove connective tissues (Bidloo, 1685; Kemp, 2010). In 1704, Ruysch injected metal into coronary vessels and used maggots and larvae to clear the soft tissues, thus obtaining a vascular cast. Subsequently use of chemicals to digest the soft tissues was employed and this was pioneered by Lieberkuhn (1711–1746), who used acid as a corrosion medium to eliminate soft tissues (Bergeron et al., 2006). Various mixtures of casting materials have since been used with different types of corrosion agents such as hydrochloric acid or potassium hydroxide (Wiltse & Pait, 1998). To date, corrosion casting is a thriving field in biomedical sciences (Bergeron et al., 2006) and museum technology (Makanya et al., 2008).

BRIEF OVERVIEW OF USES OF CASTING MATERIALS

Casting materials may be used in three different ways: (1) preparation of specimens in basic and investigative research; (2) preparation of specimens for anatomical teaching or for enhancement of skills for clinicians and surgeons; (3) preparation of museum specimens for preservation and display. The choice of the appropriate casting material is determined by a variety of factors, such as the purpose of the final casts and the kind of biological specimen or tissue involved. The possibilities range from low-viscosity, hard materials, such as mercox used for casting the tracheal system of insect species (invertebrates) (Meyer, 1989) to flexible latex rubber used for casting the respiratory system and air sacs of avian species (vertebrates) (Akester, 1960).

Casts for Teaching

Casts for anatomical courses aim to elucidate otherwise complex anatomical spatial relationships (Henry et al., 1998) and proffer 3D information not readily discernible from two-dimensional (2D) pictures or gross specimens. Such 3D replica are based on real specimens to support the learning process (Aultman et al., 2003) rather than learning from theoretical descriptions (Henry, 1992). Materials used in producing casts for anatomical studies need to be compatible with the subsequent procedures that aim to make the casts even more manageable, such as plastination or impregnation. Both flexible casting materials and hard materials are used for this process. Combined with the new anatomical preservation methods where the cadaver closely resembles the real life situation (Groscurth et al., 2001; Kerckaert et al., 2008), casting has greatly enhanced the ease of studying anatomy.

The training of new surgical approaches may require practice on isolated specimens with blood vessels cast in a

conspicuous colored material. Since freezing and thawing does not alter the consistency of soft tissue organs such as the liver, it was demonstrated that such organs combined with casting made them useful material for surgical practice (Ravnik et al., 1995). Indeed corrosion casts of livers were used to study the 3D structure of hepatic vessels and bile ducts before the surgical techniques were clinically applied (Ravnik et al., 1995). Detailed knowledge of the course, diameters, branches and possible variants of vessels is requisite in surgical organ resection and training of surgeons on such details (Kawarada et al., 2000; Onishi et al., 2000). Vessels filled with appropriate casting materials greatly enhance the visualization of their distribution. Such casts provide valuable information for many surgical fields, such as reconstructive surgery and neurosurgery for human and veterinary purposes. Vessel-filled specimens are often intended for further procedures, such as dissection, which require flexible casting materials. Aqueous solutions are also used, in particular for the visualization of vascular skin territories. The value of casts may be further enhanced by adding radiopaque materials, which allow the use of imaging techniques such as computed tomography (CT) or magnetic resonance imaging (MRI).

Casts for Research

Casts for research at the macroscopic level allow visualization of the 3D organization of structures and their topographical position in normal and pathological conditions. At microscopic and ultrastructural levels casting allows investigation of fine structures such as capillaries. At this level the viscosity of the casting materials should be such that they are capillary-passable. In the latter situation, corrosion casting and scanning electron microscopy (SEM) allow study of fine structures such as vascular beds in healthy and pathological tissues and the characteristics of vessels by analyzing the imprints of cells and nuclei left on the surface of the cast (Jamous et al., 2005; Pitynski et al., 2012). Previously we used mercox casting and SEM to demonstrate augmentation of avian renal glomeruli through intussusceptive vascular splitting (Makanya et al., 2005) as well as microvasculature expansion of avian lungs through pillar formation (Makanya et al., 2007). Casting materials used for macroscopic purposes comprise flexible and hard materials, but do not necessarily need to be capillary-passable.

Casting of Museum Specimens

Casting is one of the numerous procedures used to prepare museum specimens for preservation and presentation (Pulvertaft, 1950; Krucker et al., 2006; Makanya et al., 2008). The materials and procedures used for casting in this case depend on several factors including the preservation status of the specimen when first found and the specific purpose for which the cast is being made. Basically, where the curator has the leeway to choose and prepare specimens, all casting materials and procedures are applicable for museum specimens.

Table 1. Details of the Various Types of Casting Materials and Their General Characteristics.

Group	Description
Water-miscible materials	
India ink	Basic India ink is composed of a variety of fine soot known as lampblack, combined with water to form a liquid
Prussian blue ink	Prussian blue (ferric ferrocyanide) was one of the first synthetic pigments ever discovered. It is employed as a very fine colloidal dispersion since it is not soluble in water
Pliable materials	
Gelatin	Gelatin is a translucent, colorless, brittle flavorless solid substance, derived from collagen. It is a mixture of peptides and proteins produced by partial hydrolysis of collagen
Latex	Latex is a complex emulsion consisting of proteins, alkaloids, starches, sugars, oils, tannins, resins, and gums that coagulates on exposure to air
Silicone rubber	Silicone rubber is an elastomer (rubber-like material) composed of silicone, itself a polymer containing silicon together with carbon, hydrogen, and oxygen. Silicone rubber is generally non-reactive, stable, and resistant to extreme environments and temperatures from -55 to $+300^{\circ}\text{C}$ while still maintaining its useful properties
Hard materials	
Methyl methacrylates	Methyl methacrylate is an organic compound with the formula $\text{CH}_2 = \text{C}(\text{CH}_3)\text{COOCH}_3$. This colorless liquid, the methyl ester of methacrylic acid (MAA) is a monomer produced on a large scale for the production of poly(methyl methacrylate) (PMMA)
Epoxy resins	Epoxy is both the basic component and the cured end product of epoxy resins. Epoxy resins, also known as polyepoxides are a class of reactive prepolymers and polymers, which contain epoxide groups. They may be reacted (cross-linked) either with themselves through catalytic homopolymerisation, or with a wide range of co-reactants including polyfunctional amines, acids (and acid anhydrides), phenols, alcohols, and thiols
Polyurethanes	Polyurethane (PUR and PU) is a polymer composed of a chain of organic units joined by carbamate (urethane) links. While most polyurethanes are thermosetting polymers that do not melt when heated, thermoplastic polyurethanes are also available
Polyester resins	This is a group of unsaturated organic compounds formed by the reaction of dibasic organic acids and polyhydric alcohols. Polyester resins are thermosetting and cure exothermally. The use of excessive catalyst can, therefore, cause charring or even ignition during the curing process

DIVERSITY OF CASTING MATERIALS

In the past, many types of materials were attempted for casting and such included air, water, milk, and wax (Bergeron et al., 2006; Grabherr et al., 2007). Currently, the arsenal of materials used has been refined to a few highly effective casting media with great scientific significance as well as a strong practical impact. The casting materials used nowadays can be grouped into three categories (Table 1). These include: (1) aqueous solutions (India ink, Prussian blue); (2) flexible materials (gelatin, latexes, and silicone rubbers) and (3) hard, dimensionally stable materials (methylmethacrylates, epoxy resins, polyurethanes, and polyesters).

USES AND APPLICATION OF CASTING MATERIALS

Water Miscible Materials

Water miscible materials commonly used in casting include India ink (also known as China ink), Prussian blue ink and latex. Though latex is included here, it is best discussed under pliable materials due to its superior elastic nature.

India Ink

India ink is composed of a variety of fine soot (lampblack) combined with water to form a liquid. A binding agent such as gelatin (Zhao et al., 2004) or shellac may be added to make it more viscous and more durable once dried. India ink is easily miscible with water and has been used for angiography (Fujimoto et al., 2013), for enhancing clinical and surgical procedures (Shaari et al., 1999) and in casting (Zhao et al., 2004). The problem with aqueous solutions is their possible penetration of neighboring vascular territories via “choke vessels.” Choke vessels are arteries with reduced diameters that link adjacent vascular territories and that only dilate during times of increased demand for blood flow (Ye et al., 2012) making assessments of the exact borders of vascular territories difficult (Bergeron et al., 2006; Benzioni et al., 2009). India ink is easily miscible with other casting materials. When mixed with water, the specimen can be fixed in 8% formaldehyde (Kachlik et al., 2010) and this allows dissection.

Mixing India ink with latex (Parke, 2004; McKeon et al., 2012) improves on viscosity and pliability. Specimens filled with a mixture of India ink and latex are suited for applying the modified Spalteholz technique, which results in

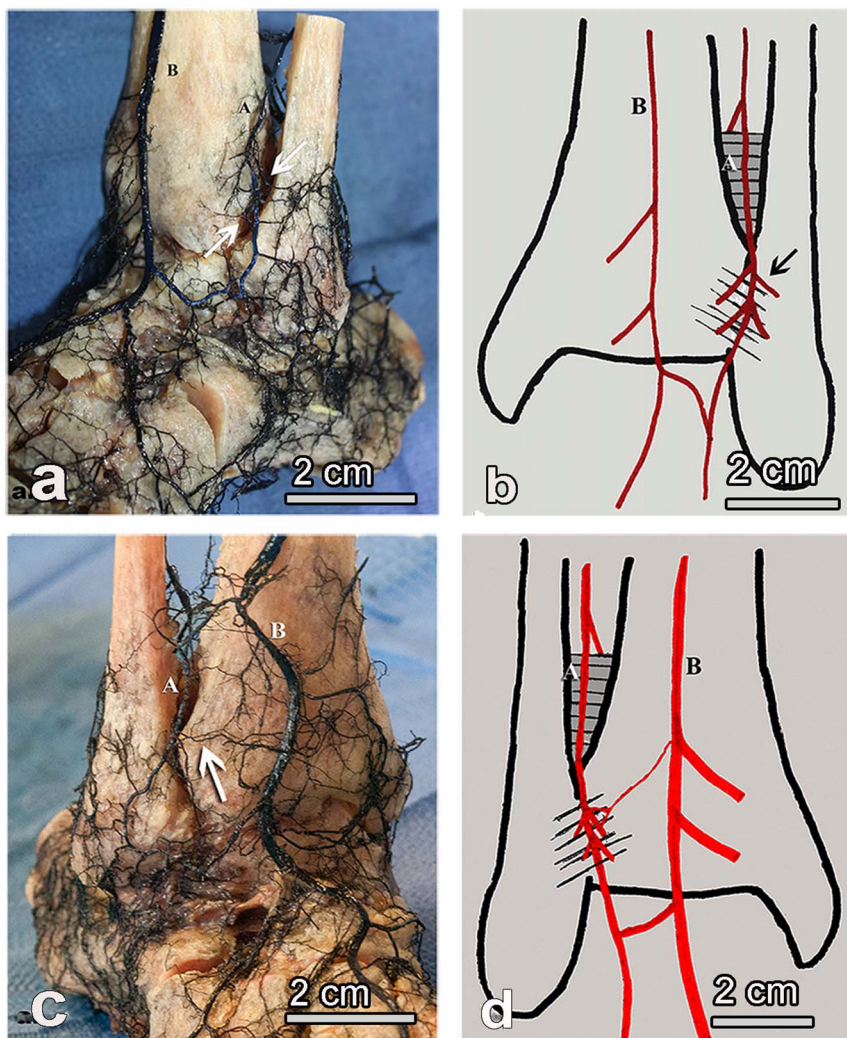


Figure 1. Photographs from India ink and latex vascular casts demonstrating the anterior type 1 and 2 modes of blood supply to the human tibulofibular syndesmosis. The schema is drawn from the casts to emphasize the details. Photograph (a) and line drawing (b) showing Anterior Type 1. In this case the anterior syndesmosis is supplied by the perforating branch of the peroneal artery (A) with multiple small branches (arrows) at the level of the syndesmosis. There is no contribution of the anterior tibial artery (B) at the level of the syndesmosis. Photograph (c) and line drawing (d) showing Anterior Type 2 mode of blood supply. The anterior syndesmosis is supplied by the perforating branch of the peroneal artery (A) with multiple small branches (arrow) at the level of the syndesmosis. The anterior tibial artery (B) also provides small branches to supplement the peroneal contribution to the anterior ligaments. Modified from McKeon et al. (2012), reproduced with permission from *The Journal of Bone and Joint Surgery*. 94, 931–938. Copyright © 2012 Wolters Kluwer Health, Inc. Scale bar is 2 cm.

a transparent specimen that allows visualization of the casted structures. Tilotta et al. (2009) used this technique to visualize the arteries supplying human auricles as did Parke on the arteries supplying the human spinal cord (Parke, 2004). As demonstrated in Figure 1, the vascular supply to the anterior tibiofibular syndesmosis in the adult human leg was visualized by first injecting India ink followed by blue latex (McKeon et al., 2012). Injection is performed in a fresh specimen followed by fixation in formaldehyde (Parke, 2004; Kachlik et al., 2010). Using this technique, it was demonstrated that the peroneal artery supplies an anterior branch (the perforating branch) to the interosseous membrane, an average of 3 cm proximal to the ankle joint. This branch provided the primary vascular supply to the anterior

ligaments in 63% of subjects studied. The anterior tibial artery provided an additional contribution to the anterior ligaments in the remaining 37% of the subjects (McKeon et al., 2012).

Prussian Blue

Prussian blue (Berlin blue) was one of the first synthetic pigments in history. It is a dark blue pigment with the idealized formula $\text{Fe}_7(\text{CN})_{18}$ and a complex composition (Berrie, 1997). It is employed as a very fine colloidal dispersion, as the compound itself is not soluble in water. One part Prussian blue ink may be diluted in five parts tap water and injected using extension tubing (Papakonstantinou et al., 2012). Dilution of the Prussian blue ink with hydrogen

peroxide (6%) produces casts with better consistency (Papakonstantinou et al., 2012).

Pliable Materials

Pliable materials are of particular importance for clinical purposes as they can be mixed with radiopaque materials enabling radiographic investigations as well as allowing further dissection (Sedlmayr & Witmer, 2002). Table 2 shows examples of applications, where radiopaque material was suspended in pliable casting materials for use in CT and MRI to produce angiograms, which enable compilation of 3D reconstructions. Common radiopaque materials are barium sulfate and lead oxide as well as iodine-containing contrast agents. Lead oxide is variously known under names such as red lead (Bergeron et al., 2006), minium (Plaisant et al., 1998), mennige, and lead (II, IV)-oxide (Thiel, 1992b). The advantage of using lead oxide as a radiopaque material is that it is cheap and easy to apply. Pliable materials have one advantage in that combined with the latest cadaver fixation techniques, they allow both anatomical dissection and training on surgical skills and the specimens can be used over and over again (Groscurth et al., 2001; Kerckaert et al., 2008).

Gelatin

Gelatin is a colorless, odorless, translucent substance derived from collagen. It requires warm conditions to maintain viscosity at a level appropriate for injection, around 40°C but not higher than 50°C, since higher temperatures result in denaturation of gelatin. The lower temperature is the limit for appropriate viscosity to reach fine vessels. Pre-warming and/or keeping the specimen in warm water enables easier injection of gelatin as was demonstrated by Bergeron et al. (2006) on whole human cadavers. Care should be taken when dealing with formaldehyde-fixed specimens since gelatin quickly hardens in the presence of formaldehyde (Tompsett, 1970; Bergeron et al., 2006). In such a situation irrigation to flush-out formaldehyde (see below) may be necessary. Gelatin can be mixed with dyes to enhance the visibility of the casted structures. It was mixed with a red dye to demonstrate the three types of vasculature on the medial aspect of the human foot (Zhuang et al., 2011). The latter technique combined fixation with casting (Fig. 2) and since gelatin is pliable, it allowed dissection of the soft tissues to enable visualization of the vessels.

Gelatin may be mixed with dyes such as methylene blue (Heymans et al., 2004). It is miscible with radiopaque materials (Table 2) such as barium sulfate or lead oxide (Bergeron et al., 2006), in which case it allows further investigative techniques such x-ray, μ CT or even MRI. A mixture of gelatin and lead oxide enabled 3D visualization of various parts of the body in a layer-by-layer transparent process using spiral CT and a special software (Tang et al., 2008). For specimens intended for plastination, gelatin is of limited value since casts end up being brittle, cracked, and stuck to the tissues subsequent to plastination (Grondin et al., 2000). Although plastination and casting may use

Table 2. Examples of Application of Casting Materials Mixed with Radiopaque Substances to Enable Various Imaging Techniques.

Casting materials	Targeted structures	Method	Specimen	References
Gelatin with lead oxide and rhodamine B	Nasal vascular network	X-ray dissection	Human cadavers	Heymans et al. (2004)
Gelatin with lead oxide and gadolinium	Vertebral venous plexus	CT; MRI	Human cadavers	Plaisant et al. (1998)
Latex and silicone rubber with barium sulfate	Cerebrovascular system	Radiographic images	Cat heads	Breit et al. (2003)
Microfil (radiopaque silicone rubber)	Cerebrovascular system	3D imaging CT; Histology	Mouse brain	Walker et al. (2011)
Silicone rubber with renografin 60 (contains 29% iodine)	Perforasomes of internal mammary artery	Spiral CT; Dissection; 3D reconstruction	Human cadavers	Zhao et al. (2004)
PU4ii diluted with 2-butanone Iopamidol	Mammary ducts	CT; 3D reconstruction	Mastectomy specimens	Twelves et al. (2012)

CT, computed tomography; MRI, magnetic resonance imaging.

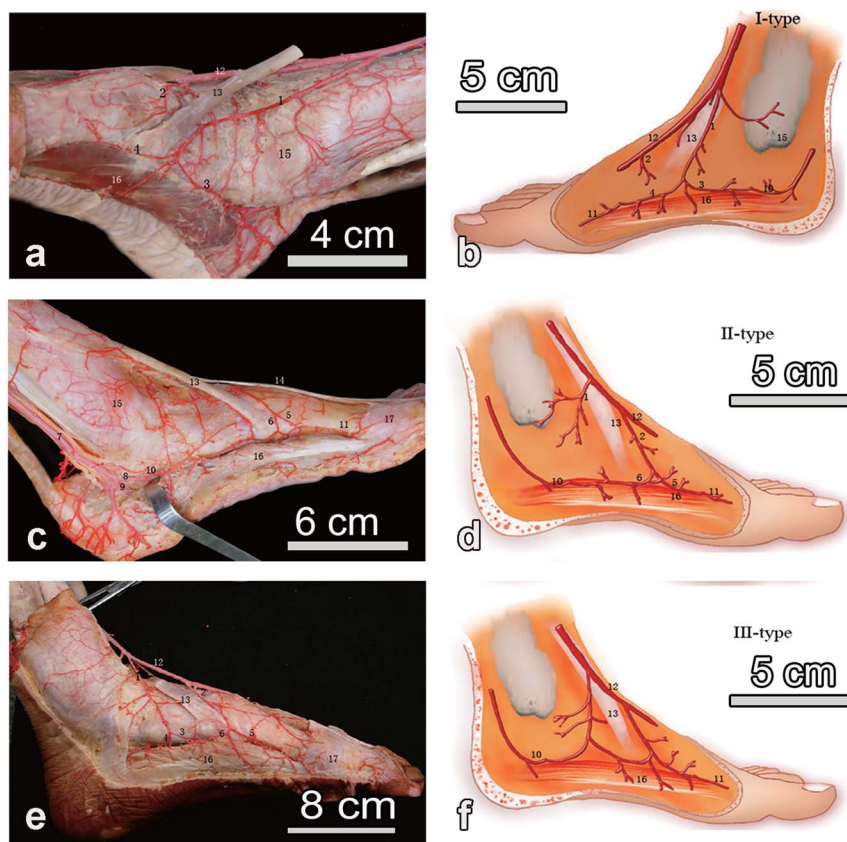


Figure 2. Three types of vasculature on the medial aspect of the human foot demonstrated using gelatin casting and explicated in the respective schema. **a, b:** Type I; **(c, d):** type II; **(e, f):** type III. The structures labeled are: 1, anterior medial malleolar; 2, medial tarsal; 3, posterior branch of the anterior medial malleolar artery; 4, anterior branch of the anterior medial malleolar artery; 5, anterior branch of the medial tarsal artery; 6, posterior branch of the medial tarsal artery; 7, posterior tibial artery; 8, medial plantar artery; 9, lateral plantar artery; 10, superficial branch of the medial plantar artery; 11, proximal superficial branch of the first plantar metatarsal artery; 12, dorsal pedal artery; 13, tendon of the tibialis anterior muscle; 14, tendon of the extensor hallucis longus muscle; 15, medial malleolus; 16, abductor hallucis; 17, first metatarsophalangeal joint. Obtained from Zhuang et al. (2011), reproduced with permission from *Plastic and Reconstructive Surgery*, 127, 1967–1978. Copyright © 2011 Wolters Kluwer Health. Scale bars: (a), 4 cm; (c), 6 cm; (e), 8 cm; (b, d, f), 5 cm.

similar polymers, the two processes are fundamentally different (Grondin, 2005). Plastination entails dehydration, saturation with an appropriate intermediary solvent, and infiltration with an in-cured polymer in vacuo (Bickley et al., 1981). The polymer replaces intracellular tissue water and lipids (Tiedemann & von Hagens, 1982) and a hollow or cavity need not be present.

Silicone Rubber Casting

Silicone rubber is an elastomer (elastic polymer) composed of silicone, itself a polymer containing silicon together with carbon, hydrogen, and oxygen. Silicone rubber is generally nonreactive, stable, and resistant to extreme environments and temperatures from -55 to $+300^{\circ}\text{C}$ while still maintaining its useful properties. It may be mixed with dyes of different colors to map out various systems in the same organ (Figs. 3 and 4) and may be combined with other procedures such as tissue fixation (Fig. 4) in which case dissection is also possible. Silicone monomer is first mixed with the dye of choice and then the silicone hardener is added before injection (Makanya & Djonov, 2008). To improve viscosity, silicone oil (polydimethylsiloxane) may be added

(Perry et al., 2000; Makanya & Djonov, 2008). Silicon rubber was used to study avian lung air conduits (Makanya & Djonov, 2008; Makanya et al., 2014) in which case its flexibility allowed for identification and counting of the various categories of secondary bronchi (Fig. 3).

Specimens exhibiting definite pathological conditions, such as obstructed airways or dilated mucous glands may be difficult to cast with silicone. Perry et al. (2000) adopted a method known as a negative-pressure casting technique to force silicone rubber into segments of pathological lungs. When mixed with radiopaque substances such as barium sulfate (Table 2), it becomes firm and allows diverse techniques such as x-ray analysis (Nöller et al., 2006) to be undertaken. The dosage of the hardener affects polymerization times and hence injection times. Kaya et al. (2006) describe a method where the mixture of silicone rubber and catalyst hardens within 1 h, which proved to be particularly useful in forensic departments since casting and autopsy procedures could be undertaken simultaneously.

Modified methods of Spalteholtz may be employed to enable tissue clearing and hence visualization of silicone

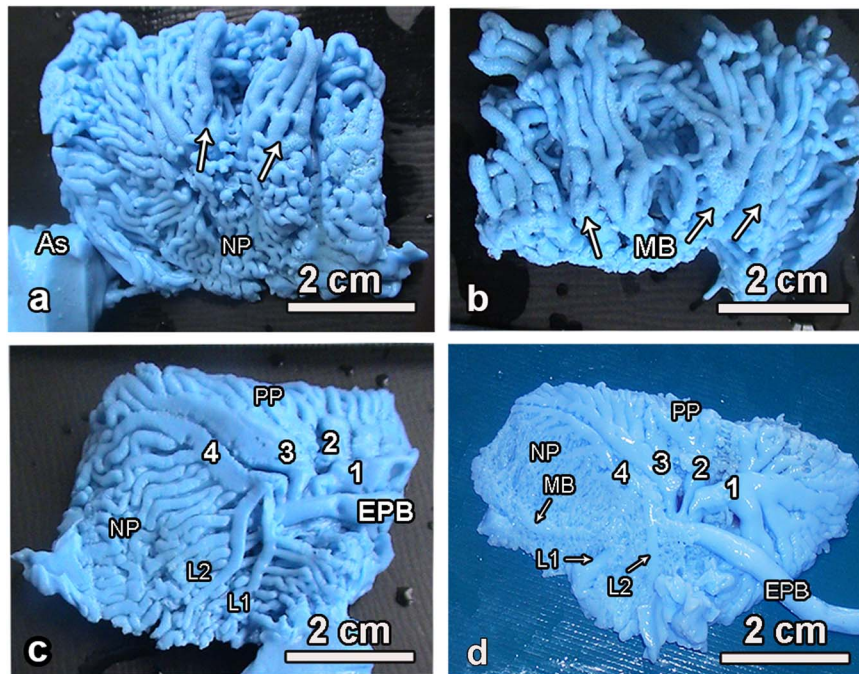


Figure 3. Macrographs of silicone rubber casts explicating the lung structure in the adult chicken (a–c) and duck (d). The laterodorsal secondary bronchi (arrows) are visible on the lateral aspect of the lung. The mesobronchus (MB) the neopulmonic region (NP) and the air sac (As) are also shown. (c, d): On the medial aspect the extrapulmonary primary bronchus (EPB), the medioventral secondary bronchi (1–4), and the lateroventral bronchi (L1 and L2 are shown). The other abbreviations are as in A and B above while PP denotes paleopulmonic region. Figures a–c are modified, reproduced with permission from *Microscopy Research and Technique*, 106, 1959–1969. Copyright © 2008 Wiley-Liss, Inc. from Makanya and Djonov (2008), while (d) is from Makanya et al., (2014). Scale bar is 2 cm.

rubber-filled vascular networks in tissues (Kappler et al., 2005). Using transparency methods and dyes can have a profound effect. Red-colored Microfil, for example, can be better seen in translucent brain tissue while yellow-colored Microfil is more prominent in translucent myocardium (Walker et al., 2011).

Silicone rubbers are suitable for subsequent postcasting procedures such as plastination as demonstrated with casts of the ventricular system (Grondin et al., 2000). Loss of color intensity during treatment in the acetone bath, a critical step in plastination, may be an undesirable outcome (Pereira-Sampaio et al., 2011). The colored pigments should be mixed with the diluent, if possible, before adding it to the casting material as recommended for silicone rubber (Smith & Rhoton, 1999).

Henry et al. (1998) produced casts of canine hearts with silicone rubber to show the overlapping spatial relationship of cardiac chambers to first year veterinary students. Having the students do the casting themselves greatly enhances understanding of the structure as was demonstrated with the constructivist approach to study cardiovascular and respiratory morphology (Hermiz et al., 2011). In the latter case, the bronchial tree and coronary arteries of rats were casted with silicone rubber by medical students, the teacher's role being only facilitative (Hermiz et al., 2011).

Latex Casting

Latex is a complex emulsion consisting of proteins, alkaloids, starches, sugars, oils, tannins, resins, and gums that coagulates

on exposure to air. Latex exists as an emulsion of polymer microparticles in an aqueous medium and is thus miscible with water. The color of latex makes it easier to identify casted structures during dissection and its elasticity helps preserve structural integrity. It occurs in a liquid form and solidifies quickly in the presence of formalin (Bergeron et al., 2006). This happens through a process called emulsion polymerization, where propagation reaction of free radicals with monomer molecules in a very large number of discrete polymer particles occurs (Chern, 2006). In standard latex casting of blood vessels, blood is flushed out and latex is injected in the arterial system and allowed to distribute for a few minutes. Subsequent injection of formalin in the venous system enhances the hardening process of the latex (Bergeron et al., 2006). Injection of formalin before latex is also used but is less reliable. An easier and more common procedure is to fix samples in formalin after casting. For example, to demonstrate radiculo-medullary arteries in human cadavers in the lumbar region (Fig. 4), samples casted with latex were kept in 20% formalin at 20°C for 2–4 weeks (Gao et al., 2013), while Nikolić and colleagues used 10% formaldehyde solution to fix latex-casted hearts (Nikolic et al., 2004).

Modifications are also possible with latex casting. For example, barium sulfate was mixed with latex to produce casts of the nasolacrimal ducts in cats without major artifacts (Breit et al., 2003), and this enabled radiography. An acceptable CT-density was achieved by mixing lead oxide (minium) with latex and for investigation using MRI, gadolinium

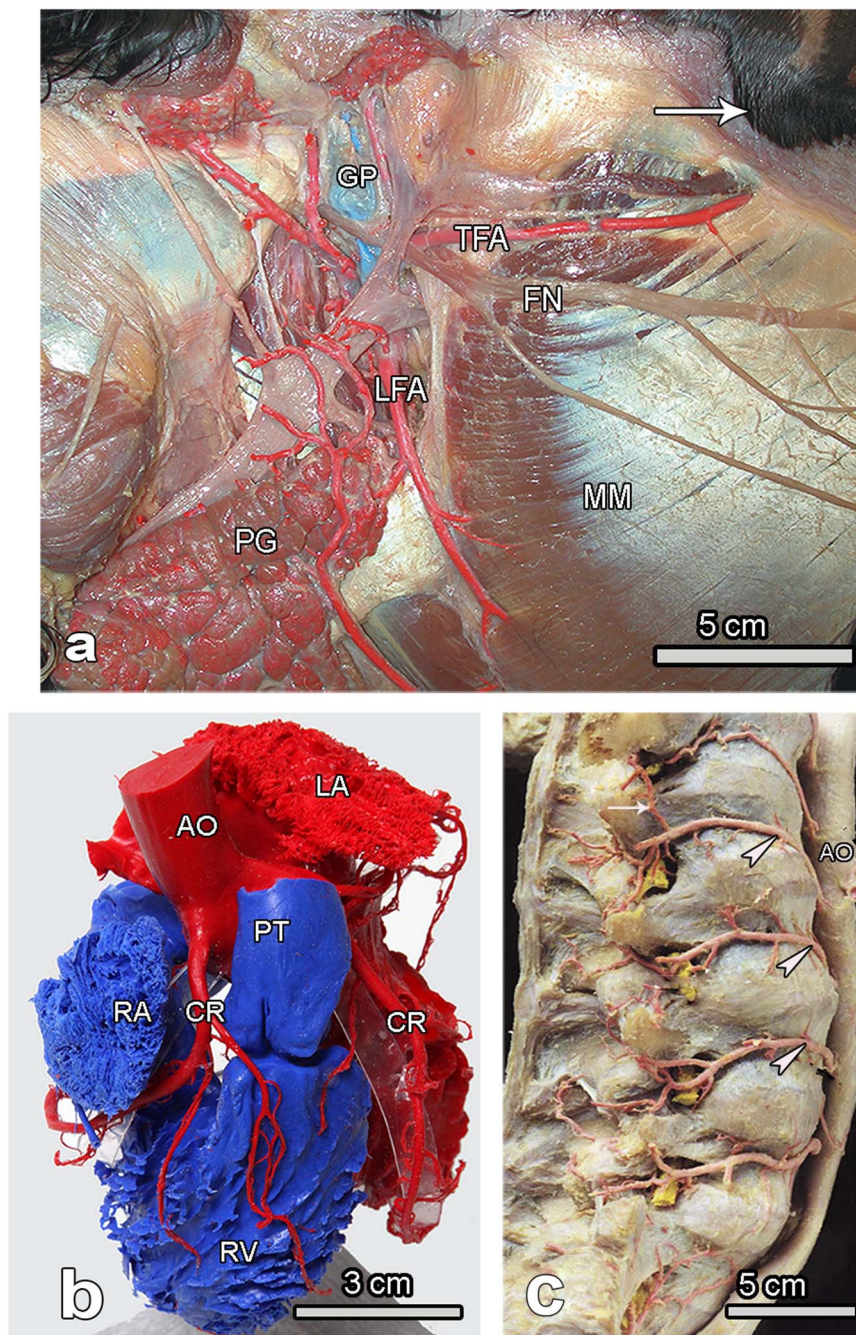


Figure 4. Macrographs demonstrating silicon casting (a, b) and latex casting (c). **a:** Fixed equine head (lateral view), with vascular silicone casting showing superficial layer of the parotid gland (PG), transverse facial artery (TFA) and the linguofacial artery (LFA). The guttural pouch (GP) has been casted with blue silicone. The facial nerve (FN) and the masseter muscle (MM) are also shown. The arrow indicates the position of the eye. **b:** Corrosion cast of pig heart, casted with blue (right heart) and red (left heart) silicone to show the coronary arteries (CR), the right atrium (RA), right ventricle (RV), pulmonary trunk (PT), left atrium (LA) and the aorta (AO). **c:** Lateral view of human lumbar spinal column showing the lumbar arteries (arrow heads) and their anastomosis. The arrow points to the anastomosis of segmental arteries in thoracolumbar region and AO denotes the aorta. **a, b:** Unpublished data from our laboratory while (c) is reproduced from Gao et al., (2013), reproduced with permission from *The Spine Journal*, 13, 966–973. Copyright © 2013 Elsevier Limited. Scale bar: (a, c), 5 cm; (b), 3 cm.

(a silvery-white, malleable and ductile rare-earth metal) was added to latex (Plaisant et al., 1998).

Viscosity of latex may be modified by addition of diluents. Diluents for latex include water (Alvernia et al., 2010), ammonium hydroxide, or triethylamine (Thiel, 1992).

Addition of diluting agents results in longer postinjection setting periods (Alvernia et al., 2010). Formalin, ethanol and diluted acid solutions cause latex to solidify (Mulisch & Welsch, 2010). Latex is available in different colors but can also be colored with poster paint or pigment paste

(Latorre et al., 2001). Here, caution is advised as the application of alternative dyes might interfere with certain steps of plastination, if such a subsequent procedure is intended. A loss of color-intensity was experienced in the smallest latex-filled structures of plastinated equine forelimbs (Latorre et al., 2001), probably because the pigment used was not color-stable. Regarding plastination, it is noteworthy that latex can swell in methylene chloride (Henry et al., 1997), which is used to saturate specimens just before *in vacuo* impregnation (von Hagens, 1979; Bickley et al., 1987). Use of acetone as a saturation medium rather than methylene chloride does not appear to engender swelling of latex (Latorre et al., 2001). Clearing, which makes the structures surrounding the cast transparent, can be achieved by treatment with glycerin, thus allowing visualization of the latex-filled arterial network within the tissues (An et al., 2012).

Hard Materials

Hard casting materials comprise methacrylates, polyurethanes, epoxy resins, and polyesters. Polyurethanes, though classified here as hard materials, can also be used as flexible casts in dissection. Both methacrylates and polyurethanes are generally capillary-passable and have been used in macroscopic as well as ultrastructural investigations.

Methacrylate Casting

Methacrylates are monomers in polymer plastics and form the acrylate polymers, which are known to be elastic, transparent and resistant to breakage. They easily form polymers because they possess very reactive double bonds in their chemical structure. The term methacrylate refers to derivatives of methacrylic acid, including the parent acid ($\text{CH}_2\text{C}(\text{CH}_3)\text{CO}_2\text{H}$), salts, esters or methyl methacrylate and the polymers of these species. Methacrylates are generally capillary-passable and have been used in both macroscopic (Fig. 5) and ultrastructural (Fig. 6) investigations. They can be mixed with dyes of varied colors to outline several different tubular systems of the same organ as demonstrated for the pig kidney (Fig. 5). Alternatively, different casting materials may be used in the same biological specimen, hence providing information of comparative value. Nöller et al. described the casting of paranasal sinuses of fresh cat heads with Technovit 7143 and Tensolciment 70. Problems occurred with both casting materials; Technovit 7143 (ratio of powder to liquid 2:1) required quick injection and the resulting casts proved to be very brittle and Tensolciment 70 seemed to be less suited for filling such large cavities. The mixture settled due to its longer curing time, which made re-injection necessary (Nöller et al., 2006).

Waldmann (2006) applied Technovit 7143 into the arteries (ratio of powder to liquid 1.7:1) and Mercor into the veins of the forelimb in common wood pigeons (*Columba palumbus*). Mercor, in contrast to Technovit 7143, was able to overcome the venous valves, thus filling even the small capillaries, which necessitated removal of the smaller vessels from the final cast, since they were not required

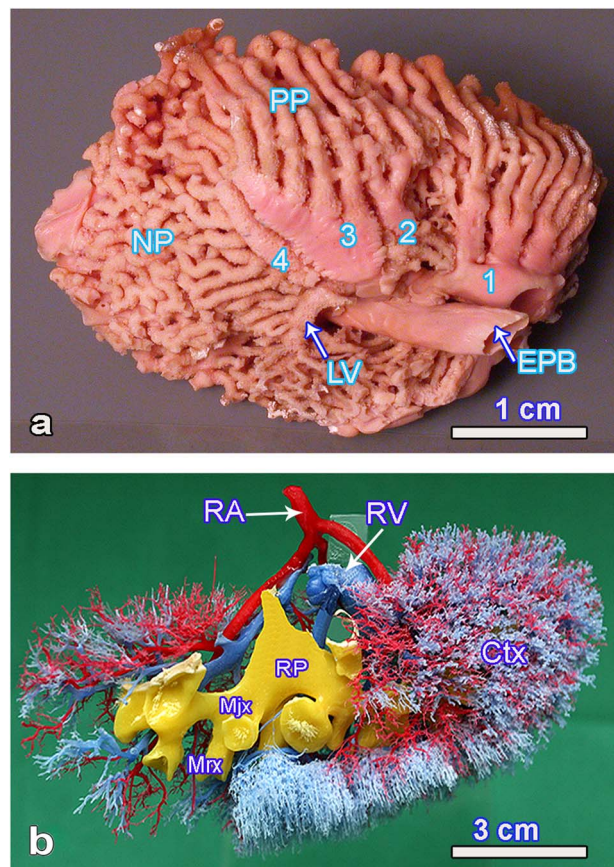


Figure 5. Technovit 3040 cast of the adult chicken lung showing the medioventral aspect. The primary bronchus (EPB), the medioventral secondary bronchi (1–4), and the lateroventral secondary bronchus (LV) are shown. Note also the neopulmonic (NP) and paleopulmonic (PP) parabronchial regions. **b:** Corrosion cast of pig kidney, produced from Technovit 7143 during student optional courses, with arteries injected with red, veins with blue and urinary system with yellow resin. The renal pelvis (RP), major calyces (Mjx) and the minor calyces (Mrx) represent the urinary system. The renal artery (RA), the renal vein (RV) and the kidney cortex (Ctx) are also shown. A is modified from Makanya and Djonov (2008). Reproduced with permission from *Microscopy Research and Technique*. 71, 689–702. Copyright © 2008 Wiley-Liss, Inc. while B is unpublished data. Scale bar: (a), 1 cm; (b), 3 cm.

(Waldmann, 2006). Technovit 7143 was observed to be brittle compared to Mercor (Waldmann, 2006). Mercor is popular for ultrastructural studies and together with SEM has been used for more than five decades (Hossler & Douglas, 2001). Its capacity to fill even the small capillaries has enabled its use in demonstration of incipient vasculature (Fig. 6) both by demonstration of new capillary sprouts (Makanya et al., 2007) as well as intussusceptive pillars (Caduff et al., 1986).

Polyurethane Casting

Polyurethane (PU, Pu4ii) is a polymer made up of a chain of organic units joined by urethane links. Polyurethane polymers are formed by reacting an isocyanate ($\text{R}-\text{N}=\text{C}=\text{O}$) with a polyol. Both the isocyanates and polyols

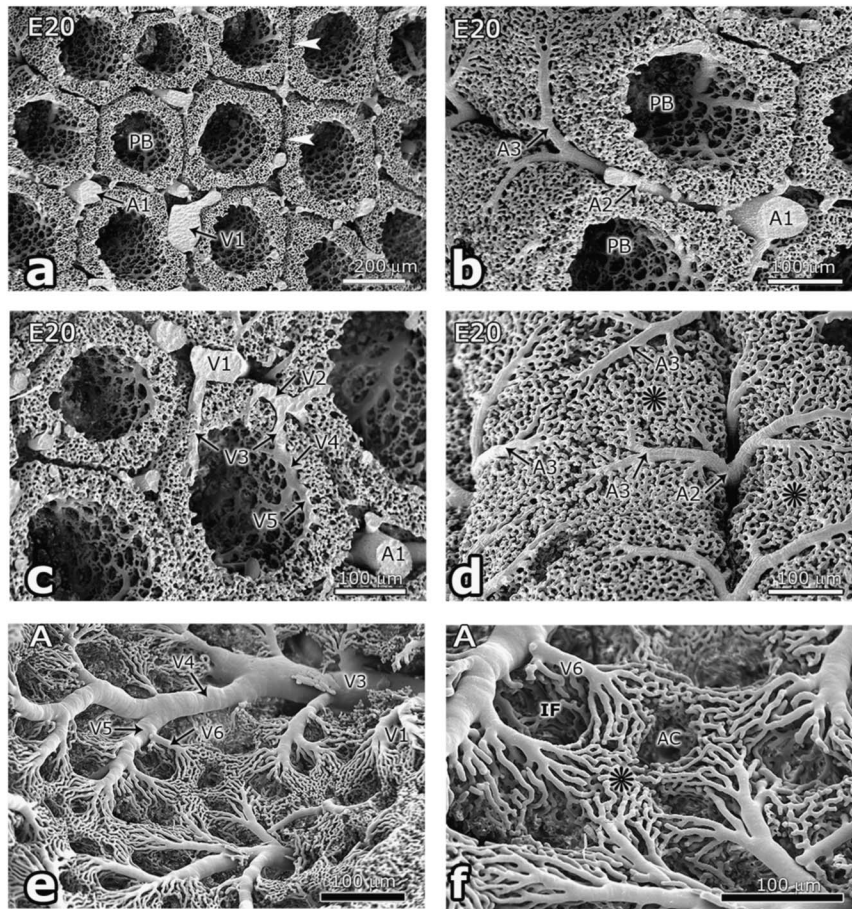


Figure 6. SEM micrographs from mercox vascular casts of the chicken lung showing the arrangement of the parabronchial (PB) vessels in the day 20 (E20) embryo (a–d) and in the adult (e, f). A: the capillary meshwork forms hexagonal tubes that surround the PB lumen. Adjacent PB are separated by the interparabronchial septum (arrowheads), which contains the interparabronchial artery (A1) and the interparabronchial vein (V1). b: A1 gives rise to parabronchial arteries (A2), which form parabronchial arterioles (A3) that break into a dense capillary meshwork. (c): V1 runs parallel to A1 and is joined by the parabronchial veins (V2), which receive intraprabronchial veins (V3). Atrial veins (V4), formed from convergence of septal venules (V5), drain into V3. D: on the external aspect of the capillary meshwork A3 branch from A2, and give rise to blood capillaries (asterisks) that surrounds the air capillaries (ACs). e, f: In the adult lung, infundibulae (IF) are surrounded by infundibular venules (V6), and join septal venules (V5) directly, which lead into atrial veins (V4). V4 drain into V3. Notice an individual AC surrounded by blood capillaries (asterisk). Reproduced with permission from *Journal of Applied Physiology*. 106, 1959–1969. Copyright © 2009 the American Physiological Society. Scale bar: (a), 200 μm ; (b–f), 100 μm .

(alcohols with multiple hydroxyl groups) used to make polyurethanes contain an average of two or more functional groups per molecule (Santerre et al., 2005). Polyurethanes remain one of the most popular group of biomaterials applied for medical devices due to their segmented block copolymeric character, which proffers them with a wide range of versatility in terms of tailoring their physical properties as well as blood and tissue compatibility (Santerre et al., 2005). In casting, PUs are versatile in that they can be used as pliable materials to cast specimens for dissection courses, as corroded casts for display, or even as casts for SEM (Figs. 7, 8). Indeed using polyurethane casting, it was demonstrated that glomerular recovery after Thy1.1 nephritis proceeded through intussusceptive angiogenesis (Wnuk et al., 2011, 2012), a novel mechanism of angiogenesis that is also involved in vascular patterning and angioadaptation (Makanya & Djonov, 2009; Makanya et al., 2009). A combination of relative hardness and

elasticity makes PU4ii a superior casting agent compared with silicone (Meyer et al., 2007). Dissection of vessels injected with PU4ii proved easy and the vessels were palpable within the surrounding tissue, but nevertheless were elastic and tear-resistant (Meyer et al., 2007).

Polyurethanes are suitable for a wide range of purposes, from macroscopic (Fig. 8) to ultrastructural investigations (Hlushchuk et al., 2008, 2011). By applying the Spalteholz technique, arterial anastomoses can be demonstrated within tissues as described for the latissimus dorsi muscle (Meyer et al., 2007). The viscosity can be lowered by adding ethyl methyl ketone (EMK) or dimethylketone. Dilutions with 30% EMK showed no significant changes in shrinkage behavior and other properties (Meyer et al., 2007). Polymerization times can be prolonged by using a slower hardener (Krucker et al., 2006; Meyer et al., 2007). The radio-opaque Iopamidol, a nonionic, low-osmolar iodinated contrast agent can be mixed with PU4ii

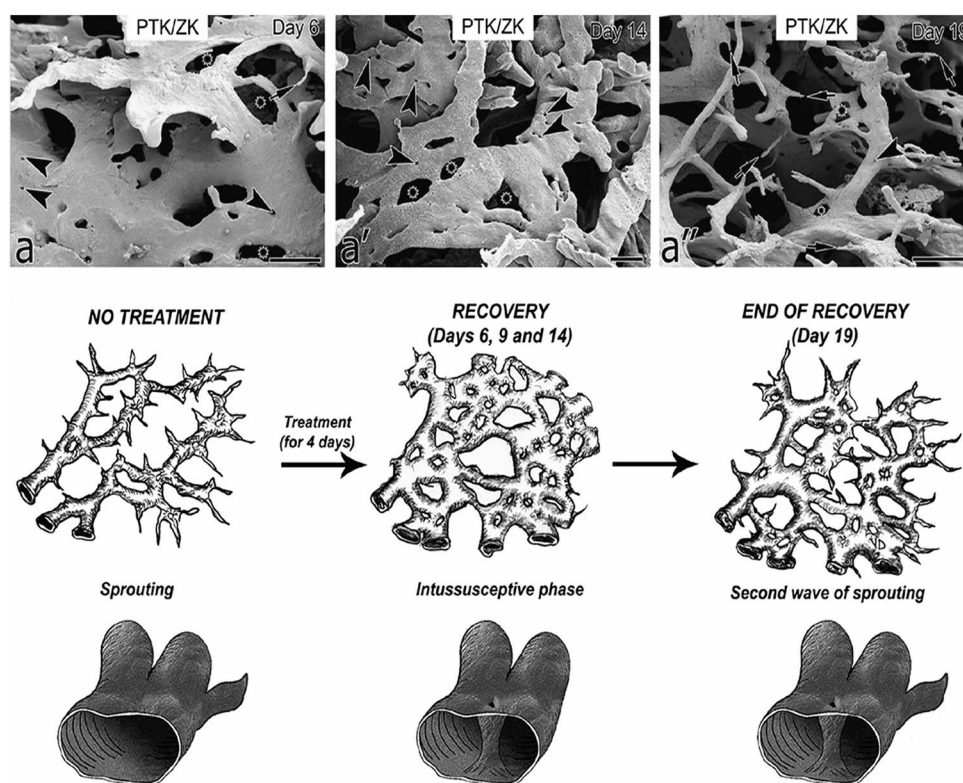


Figure 7. Time-course of the angiogenic switch from sprouting to intussusception during tumor recovery. **a:** Scanning electron micrographs representing the vascular pattern of the PTK/ZK treated tumors on day 6 (a), 14 (a') and 19 (a''). On day 6 and 14 pillars (arrowheads) and meshes (asterisks) dominate. In contrast to day 6 and 14, on day 19 (a'') the sprouts (arrows) prevail in the vascular pattern of the PTK/ZK-treated tumors with some pillars (arrowhead) or meshes (asterisks) present. The scheme in (B) represents the above-described changes: in non-treated tumors the dominant mode of angiogenesis is sprouting. After the short-term therapy intussusception prevails: so called, intussusceptive phase of recovery (days 6, 9 and 14). On day 19 the second wave of intussusception occurs, what is characterized by numerous sprouts along with pillars or meshes. Adapted from Hlushchuk et al. (2011), reproduced with permission from *The International Journal of Developmental Biology*, 55, 2011, 563–567. Copyright © 2011 UBC Press. Scale bar is 100 μm .

for CT analyses (Twelves et al., 2012), although casts from PU4ii can be imaged using μCT without contrast agents to produce digital 3D reconstructions. Fluorescent colors can be added to PU4ii, which already possess inherent fluorescent properties, enabling the use of confocal microscopy (Meyer et al., 2008). Histological sections can be prepared from tissues in which blood vessels have been cast with PU4ii (Meyer et al., 2007), which gives the possibility that other techniques such as immunostaining are feasible.

Epoxy Resin Casting

Epoxy resins (polyepoxides) are a class of reactive prepolymers and polymers, which contain epoxide groups. They may be cross-linked either with themselves through catalytic homopolymerization, or with a wide range of co-reactants (hardeners). The hardeners include polyfunctional amines, acid phenols, alcohols, and thiols (Tanaka, 1988). The hardeners are often referred to as curatives, and the cross-linking reaction is commonly referred to as curing. Reaction of polyepoxides with themselves or with polyfunctional hardeners forms a thermosetting polymer, often with strong mechanical properties as well as high temperature and chemical resistance (Tanaka, 1988).

Epoxy resin (aradite) was used to study ischemia and neovascularization in the rat hind limb (Fig. 9) with the observation that collateral vessel formation and recanalization of previously thrombosed arteries occurred (Westerweel et al., 2005) to restore blood circulation in the limb. The latter authors combined epoxy resin casting with the Spalteholz technique, which entails dehydrating tissues in ascending concentrations of ethanol and clearing with methyl benzoate (Spalteholz, 1914; Westerweel et al., 2005).

Biodur E20 is a translucent epoxy resin of medium viscosity, with firm, slightly flexible consistency. It was used for casting the arteries in the periodontium of horse teeth, some of which showed the quality required for SEM analysis. This was achieved by lowering the viscosity using MEK (Masset et al., 2006). The polymerization time for epoxy resins can be prolonged by lowering temperatures (6–8°C) (Riepertinger & Heuckendorf, 1993). The filling of the arteries supplying delicately soft tissue such as the brain or casting the brain ventricular system is usually preceded by stabilizing the tissue with chemical fixatives (Aultman et al., 2003). Stabilization of the brain *in situ* before casting with Biodur E20 was achieved with 10% formalin fixation for about 3 h before injection with the epoxy resin. The brain

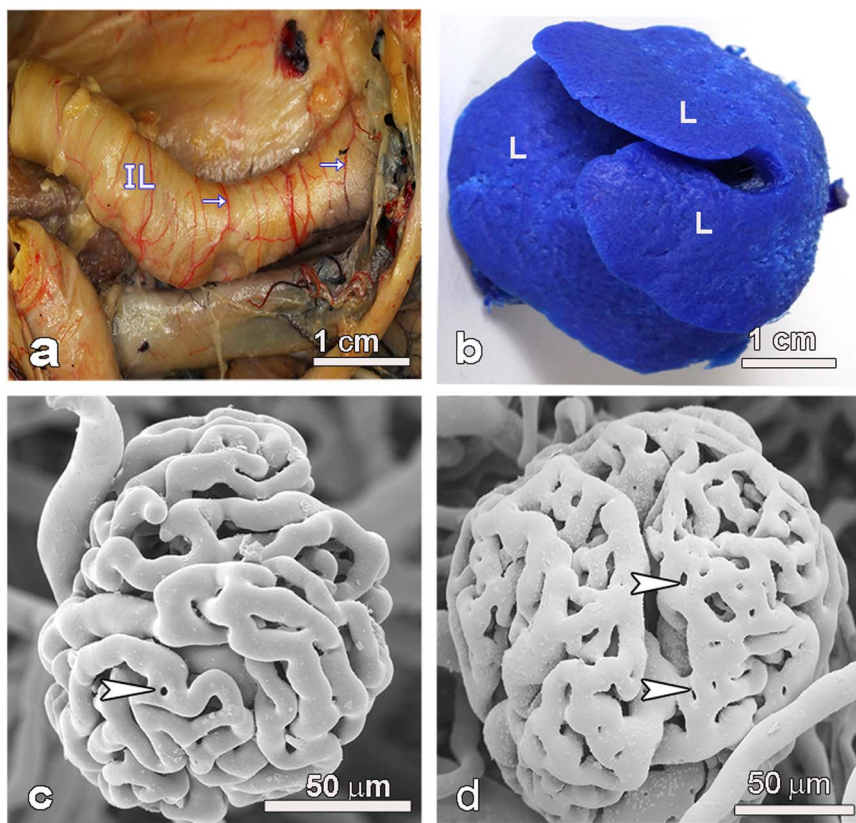


Figure 8. Photographs showing the various applications of polyurethane. **a:** Pu4ii mixed with red dye to show vasa vasorum (arrowheads) in the human iliac artery. **b:** Pu4ii mixed with blue dye to cast a mouse liver. **c, d:** SEM micrographs of polyurethane casts showing a normal glomerulus (**c**) and one that is recovering from Thy1.1 nephritis (**d**). Note that restitution of blood vessels in this case proceeds through intraluminal pillar formation (arrowheads). NB: **a–d:** are unpublished data kindly donated by colleagues Hannes Bangerter and Monica Wnuk. Scale bar: (a, b), 1 cm; (c, d) 50 μm .

specimen was subsequently plastinated for use by medical students (Riepertinger & Heuckendorf, 1993).

Polyester Resin Casting

The polyester resins used in casting are unsaturated resins formed by the reaction of dibasic organic acids and polyhydric alcohols. Water, a by-product of esterification reactions, is continuously removed driving the reaction to completion. The use of unsaturated polyesters and additives such as styrene lowers the viscosity of the resin. The initially liquid resin is converted to a solid by cross-linking chains (Stamatakis et al., 2010). Creation of free radicals at the unsaturated bonds propagates a chain reaction to other unsaturated bonds in adjacent molecules, linking them in the process. To reduce working temperatures (called cold-setting) and thus hasten the hardening process a catalyst is used (John et al., 2008). Three percent methyl ethyl peroxide (Pereira-Sampaio et al., 2012) or styrene monomer (John et al., 2008) are popular catalysts. Polyester forms a brittle nonpliable cast, is capillary-passable, can be used for SEM (Ohkuma et al., 1997), and is also used as a plastination resin (Weiglein, 1997). Excessive catalyst may also cause the product to fracture or form a rubbery material.

GENERAL PROCEDURES FOR PRODUCING CASTS

The general procedures for producing good casts are similar for all materials and purposes. Three main steps, which might differ in the amount of effort and time required are recognized. These include the preparatory process, injection, and the postprocessing work. Handling of the casting materials, particularly, the toxic solvents and catalysts requires strict adherence to instructions and safety precautions. For all materials the procedures and attendant precautions are normally provided by the manufacturers and are included in the consumer brochures.

Precasting Procedures

Preparatory work includes the handling of the biological specimen selected for casting and the preparation of the casting material. The success of the cast also depends on the biological specimen involved, such as its age (Sanan et al., 1999) and condition. In general, wherever possible, it is advisable to use fresh tissues to circumvent problems, such as rigor mortis, postmortem degradation of the vascular system, or pressure sores (Bergeron et al., 2006). Nevertheless, cattle feet stored for 2–3 weeks at 4°C revealed better filling at

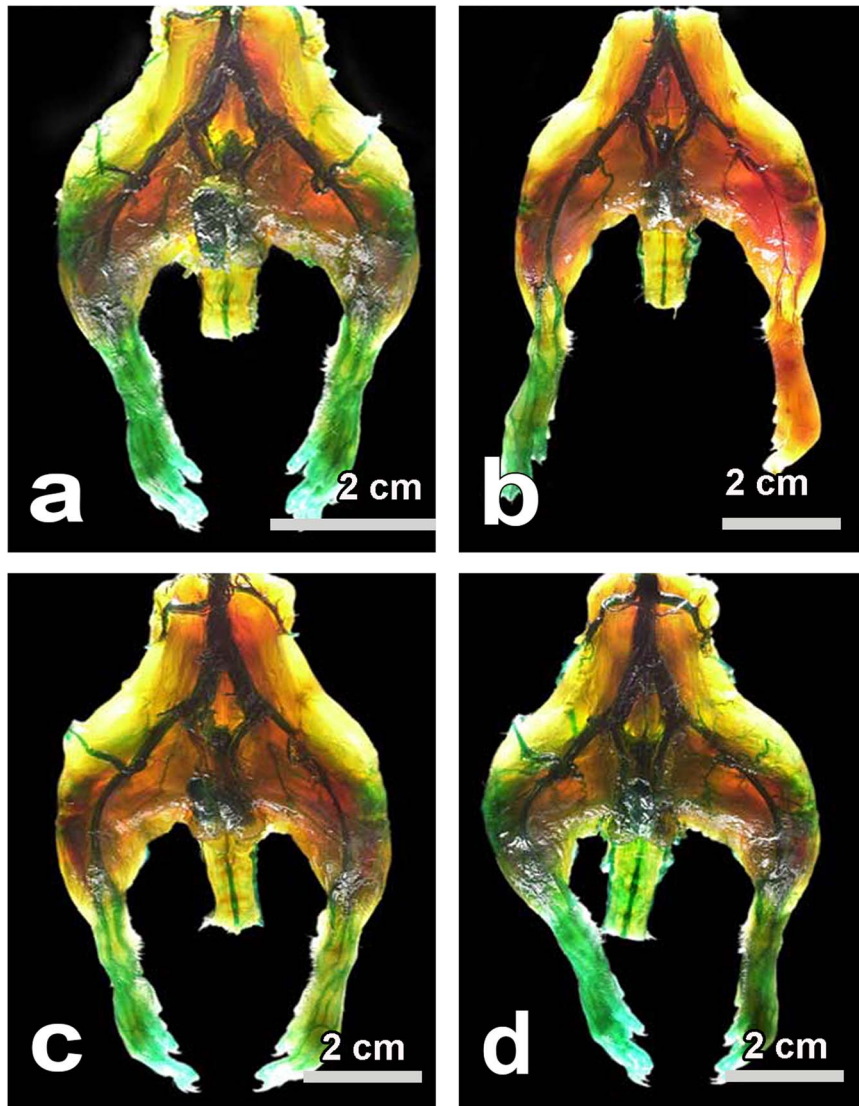


Figure 9. Series of araldite preparations photographed from the ventral side, made in a control rat (a) and after induction of ischemia by ligation of the left femoral artery. Seven days after ligation (b), there is profound ischemia on the ligated side, and distal arteries are thrombosed. After 14 days (c), collaterals have formed and flow is restored in the original conduit arteries. Total recovery has taken place after 21 days (d). Modified from Westerweel et al. (2005), reproduced with permission from *Cardiovascular Pathology*, 14, 294–297. Copyright © 2011 Elsevier Limited. Scale bar is 2 cm.

the microvascular level when compared with fresh specimens. It appears that the time lag had resolved postmortem blockages without disrupting the vessels as confirmed by SEM (Hirschberg et al., 1999).

Generally, prior embalming results in tissue fortification allowing easy handling and ample time for casting, especially for Thiel embalming (Graz method) where specimens are preserved in near natural color, strength and pliability (Jaung et al., 2011). Such specimens are particularly suitable for surgical courses, as specimens remain soft and flexible and the colors resemble those of real patients (Jaung et al., 2011; Thiel, 1992a). Latex and silicone rubbers can also be applied in formalin-embalmed specimens as demonstrated for facial arteries (Magden et al., 2004; Pinar et al., 2005). The arteries supplying isolated brains were demonstrated with latex casting, subsequently immersion-fixed in 10% formalin

(Sen et al., 2011). Latex hardens quickly in the presence of formaldehyde (Bergeron et al., 2006), ethanol and diluted acids (Mulisch & Welsch, 2010). Pu4ii casting can also easily be applied to formalin-embalmed specimens (Meyer et al., 2007).

Dissection and Irrigation

Where small components of a specimen are targeted, dissection may be required to reveal the supplying vessels for injection (Alvernia et al., 2010) or where specific organs such as the kidney need to be isolated for individual casting. Irrigation of the vasculature of fresh specimens is done with phosphate buffered saline, preferably mixed with an anticoagulant such as heparin to prevent blood from clotting (Makanya et al., 2007). In fixed specimens, irrigation with

tap water is done to remove blood clots and tissue debris and also to reveal the anatomical variants of the arterial and venous systems (Alvernia et al., 2010). Depending on the type of specimen under investigation, it may be necessary to irrigate the arterial and venous systems separately. In the latter circumstance, the arterial system is irrigated before the venous system because venous congestion may lead to increased resistance and decreased arterial flow (Alvernia et al., 2010). Filling of the targeted body cavities for casting may require the insertion of cannulas. Different kinds of cannulas can be used (Sanan et al., 1999), for example Foley catheters, which allow the inflation of a balloon to close the vessels following injection (Alvernia et al., 2010).

Irrigation of vessels is subject to controversy as previously noted (Grabherr et al., 2007). Some authors describe it as an essential step to remove blood clots and possible remains of embalming fluid (Sanan et al., 1999). Others achieved their best casts from nonrinsed specimens. For example, rinsed cattle feet prepared for casting with Mercox or Tensolcement revealed more filling defects when compared with nonrinsed feet (Hirschberg et al., 1999). A possible explanation might be that the perfusion solution provided additional blockage finally preventing capillary filling (Hirschberg et al., 1999). Nevertheless, one advantage of irrigation is the detection of leaks (Shimizu et al., 2006) and the opening of possible anastomoses (Alvernia et al., 2010). Pre-fixation of tissue is recommended for brains to provide organ stability (Riepertinger & Heuckendorf, 1993; Aultman et al., 2003; Alvernia et al., 2010) and to improve vascular properties before casting the microvasculature (Verli et al., 2007). For small specimens with delicate blood vessels such as those of the area vasculosa, irrigation or even canulation may not be feasible and the resin is injected directly (Dimova et al., 2013). In the latter situation it is mandatory that the vascular system is opened at an appropriate point to allow escape of the displaced blood.

Evacuation

In plastination, evacuation refers to removal of vaporized acetone from tissues to allow infiltration with the desired polymer resin, a process conducted *in vacuo* (Ravi & Bhat, 2011). In casting, evacuation may refer to removal of air from the casting medium or from the specimen to be casted. In the former case, the process of evacuation is an important step and aims to remove any trapped inclusions of air from the mixture as air bubbles might result in incomplete casts prone to fracture (Leiser & Pfarrer, 2005). Short polymerization times might prevent this as seen with Technovit 7143 (Cordes, 1989). Evacuation may also refer to expulsion of air particularly from blind ending cavities such as the tracheobronchial system of lungs (Jacob et al., 2013). This is achieved either by removing lungs from the thoracic cavity (Cope, 2008) or inducing a pneumothorax by puncturing the diaphragm, or by making an opening in the thoracic cage (Maina & van Gils, 2001; Makanya et al., 2001).

Injection and Infusion Procedures

In general, injection and infusion pressures need to be carefully adapted to prevent either incomplete filling or rupture of structures (Verli et al., 2007). Overfilling of the arterial systems might result in leakage of casting material into the venous system (Bergeron et al., 2006). Many authors describe the manual injection of casting material but the use of an injection apparatus has advantages as it keeps a constant injection pressure and can help to prevent the introduction of air bubbles (Minnich & Lametschwandner, 2010). There are several signs that mark the end of the injection process, such as the outflow of capillary-passable casting materials from the venous side (Pollitt & Molyneux, 1990; Leiser & Pfarrer, 2005; Alvernia et al., 2010). Outflow from contralateral arteries (Sanan et al., 1999) or opposite ends of ducts as well as casting material that shines through the skin (Vandeghinste et al., 2011) are positive signs, which are often preceded by an increase of pressure within the syringe (Latorre et al., 2001). The complete emptying of the syringe is not recommended as it might introduce air bubbles (Cordes, 1989). Tissue damage from pressure points can be avoided by injecting the casting material while the specimen or organ is floating (Cordes, 1989) or by transferring it into a water tank immediately following injection (Bergeron et al., 2006). To fill more than one artery such as the simultaneous injection of the four main arteries of the brain can be realized by using a four-way injection system (Riepertinger & Heuckendorf, 1993). In partially blocked tubular systems, application of a negative pressure helps infusion of the casting material. Perry et al. (2000) used 10 kPa negative pressure generated in a vacuum chamber to force silicone rubber thinned with silicone oil into lung tissue with partially blocked airways.

Postcasting Procedures

The procedures immediately subsequent to casting depend on the targeted final product. For specimens intended for anatomical and surgical procedures, further dissection may be necessary. Tissue clearing is done making the specimen transparent if the casted internal structures are intended for display *in situ* (Spalteholtz, 1914; Walter et al., 2012). Corrosion of surrounding tissue is undertaken if clean casts devoid of any tissue are required and in addition sputter-coating with metals or metal complexes is done if specimens are intended for observation with a SEM (De Sousa Bolina et al., 2013). It is essential to await the complete polymerization of the casting material, which ranges from hours to days and which is dependent on the diluting agents used.

Dissection

Many researchers and surgeons appreciate the advantages of filled and colored vessels as they facilitate dissection and identification of vessels, their course and branches, and the determination of vessel diameters (Perry et al., 2000; Magden et al., 2004; Kappler et al., 2005; Pinar et al., 2005; Coquerel-Beghin et al., 2006). Dissection may be done post

casting either to remove undesirable tissue such as fat or to reveal targeted structures such as blood vessels (Gao et al., 2013).

Tissue Clearing

A 3D visualization of the vascular network within organs is possible by applying a technique that makes the organ translucent, such as achieved with the method according to Spalteholz (Tilotta et al., 2009). It is noteworthy that the method is time-consuming and only applicable on small specimens and, of course, is most effective with filled vessels (Bergeron et al., 2006). Modified methods of Spalteholz were shown to enhance visualization of silicone rubber-filled vascular networks in muscles (Kappler et al., 2005) and PU4ii-filled arterial anastomoses within parts of the latissimus dorsi muscle (Meyer et al., 2007). Clearing agents include glycerin (An et al., 2012), solution of benzyl alcohol, benzyl benzoate (Walter et al., 2012), or methyl benzoate (Westerweel et al., 2005). One negative aspect, possibly resulting from the clearing process, might be the swelling of vessels as observed by Meyer et al. (2007).

Corrosion

The removal of soft tissue post casting can be biological or can employ chemical methods. In nonfixed/nonpreserved tissue, the natural processes of autolysis and putrefaction, which utilize endogenous and microbial enzymes, respectively, ultimately result in tissue destruction (Culling, 1974). This process can take a long time and is aesthetically undesirable. Use of carrion insects (Braack & de Vos, 1987) is more elegant since such are easier to control.

The choice of corrosive agents depends on the purpose of the cast and on the properties of the tissue. Preservation of delicate structures requires mild methods of maceration, such as enzymatic maceration, long-term natural decay of organic material or the use of biological methods (Cordes, 1989). For example, the larvae of necrophagous hide beetles (*Dermestes maculatus*) were used to clear the soft tissue from silicone rubber-filled nasolacrimal ducts of cats as well as the nasal cavity (Nöller et al., 2006). In a duck lung specimen casted with silicone rubber and stored for the rubber to cure, we found hordes of maggots from houseflies (*Musca domestica*) devouring the soft tissues 3 days later and let them complete the process. A time-consuming (about 6 months) but effective method is the use of common detergent at 60°C (Masset et al., 2006). Fresh biological material can be boiled in water for several hours, which macerates the soft tissue as was demonstrated in silicone rubber-filled lungs and airways (Henry, 1992). Other methods include the use of chemicals such as strong alkalis, Ariel, or enzymes such as pancreatin or papain (Pulvertaft, 1950). Different dilutions of potassium hydroxide (KOH) have been used for a wide range of specimens and for various durations as the following examples show: KOH (20%) at 40°C for 12 h applied on Technovit 7143 and Mercor-filled vessels (Waldmann, 2006); 40% KOH at 30°C for 1 day applied on

Technovit 7143-filled ovine livers (Cordes, 1989); 7.5% KOH at 50°C for 24 h applied on PU4ii-filled mouse arteries (Krucker et al., 2006). Other investigators (Hossler et al., 2013) used 5% KOH at 60°C overnight showing that at higher temperatures, a lower concentration of the corroding reagent is feasible. The duration of the corrosion process depends on how tough and big the soft tissue is and may require up to 4 weeks.

SEM

Casts intended for use in SEM need to be processed according to the general procedures stipulated for SEM (Worthen & Wickham, 1972; Verli et al., 2007). Bony structures, for example can be decalcified using formic acid to reveal the casts for further processing (Krucker et al., 2006). Further steps comprise dehydration, drying and mounting of the specimen on a conductive surface and sputter coating with a suitable metal or metal alloy such as chromium, gold or palladium, and finally adjusting the acceleration voltage during microscopy (Verli et al., 2007). The application of μ CT requires the immersion of the specimens in osmium as demonstrated for PU4ii (Meyer et al., 2007) or Batson No.17 (Vandeghinste et al., 2011). Morphometric techniques such as estimation of vascular density (Makanya et al., 2007) or branching angles (Ackermann et al., 2013) can be determined from casts imaged by SEM.

Plastination and Polyethylene Glycol (PEG)

Impregnation

Some casting materials are of limited suitability for plastination, such as seen with gelatin that produces cracks (Grondin et al., 2000) and latex that can swell in methylene chloride (Henry et al., 1997). For application of plastination as a postcasting procedure, epoxy resins such as Biodur E20 and silicone rubbers, such as Biodur S14 are recommended. It is advisable to test the colors beforehand (Pereira-Sampaio et al., 2011). Silicone rubber casting is compatible with subsequent PEG impregnation (Bertagnoli et al., 2012).

METHODS FOR MODIFICATIONS OF CASTING PROCEDURES

The modifications most often applied affect viscosity and the time required for polymerization. The viscosity of a casting material refers to its fluidity, whether it is thick (high viscosity) or thin (low viscosity), and hence determines its flow through the varying diameters of structures. Polymerization time, sometimes referred to as pot life, determines the time left from mixing the components, including any additives and hardener (catalyst), until the mixture starts to cure. Viscosity needs to be modified in the uncured state and, in turn, affects the time needed for polymerization. Common diluting agents and other means of influencing viscosity and polymerization times are provided below.

Gelatin

Gelatin requires warm conditions to maintain viscosity at a level appropriate for injection, around 40°C but not higher than 50°C (Bergeron et al., 2006). Lower temperatures cause it to solidify but the process is reversible. The protein content affects setting times. Gelatin quickly hardens in the presence of formaldehyde (Tompsett, 1970; Bergeron et al., 2006).

Latex

Latex can be diluted with water (Alvernia et al., 2010), ammonium hydroxide or triethylamine. The addition of diluting agents results in longer postinjection setting periods (Alvernia et al., 2010). Formalin, ethanol, and diluted acid solutions cause latex to solidify (Mulisch & Welsch, 2010).

Silicone Rubbers

Silicone oils (polydimethylsiloxane) are used to dilute silicon rubber (Perry et al., 2000; Makanya & Djonov, 2008) but thinner solutions are also used (Sanan et al., 1999). The ratio (silicone rubber: thinner) should be adapted to the type of vessel involved. Sanan et al. (1999) recommend a less viscous mixture for the arterial system due to the greater resistance and mixed 1:1 for arteries and 2:1 for veins, but a wide range of ratios has been used by others (Shimizu et al., 2006). Adjusting the dosage of hardener usually alters polymerization times (Kaya et al., 2006).

Methacrylates

Technovit 7143 can be diluted by varying the ratio of powder to liquid or by adding monomer methyl methacrylate (MMA), which acts as a diluent (Hirschberg et al., 1999) or acetone (Cordes, 1989). Polymerization times can be prolonged by modifying the ratio of powder to liquid or by storing it in a refrigerator (6–8°C) (Hirschberg et al., 1999), although Waldmann (2006) observed only slight improvement with injection of Technovit 7143 into ice-cooled specimens.

Tensolcement can be diluted with ethyl acetate (Hirschberg et al., 1999) while Mercoc (Hirschberg et al., 1999) and Batson No.17 (Pollitt & Molyneux, 1990) are diluted with the monomer, MMA. Polymerization durations for Tensolcement and Mercoc can be prolonged by lowering temperatures (6–8°C) (Hirschberg et al., 1999). Polymerization of Batson No.17 begins within 3–5 min of mixing with the hardener and thus requires quick injection (Leiser & Pfarrer, 2005), cooling down (4°C) delays the process (Leiser & Pfarrer, 2005). Reducing the amount of accelerator in the latter case prolongs the polymerization process (Pollitt & Molyneux, 1990). The addition of diluent acetone causes Technovit 7143 to become brittle and to trap more air bubbles (Cordes, 1989).

Polyurethanes (PU4ii)

Polymerization times can be prolonged by using a slower hardener (Krucker et al., 2006) and viscosity can be lowered with EMK or dimethylketone (Meyer et al., 2007). Any modification

of viscosity comes at the expense of other desired properties, such as curing times. Meyer et al. (2007) diluted PU4ii with maximal 30% MEK without noticing significant changes in shrinkage behavior and other properties, but as noted elsewhere, shrinkage is a well-known problem associated with dilution (Verli et al., 2007).

Coloration

Desired modifications of properties comprise coloration as it facilitates visualization and can make a difference in distinguishing tissues. Red-colored Microfil, for example, can be better seen in translucent brain tissue while yellow-colored Microfil is better visible in translucent myocardium (Walker et al., 2011). Possible color inducing agents include colored dyes, pigments, and color pastes and in principle it is possible to apply any color that mixes well with the casting material (Nöller et al., 2006; Shimizu et al., 2006). Latex is available in different colors, but its color can be altered by adding poster paint or pigment paste (Latorre et al., 2001). Here, caution is advised as the application of alternative colors might interfere with postprocessing steps, such as plastination. Addition of pigmented colors to silicone rubber resulted in a loss of color intensity during treatment in an acetone bath (Pereira-Sampaio et al., 2011) and in latex-filled structures of plastinated equine forelimbs (Latorre et al., 2001). A possible explanation is that the pigment used was not color-stable (Latorre et al., 2001). The colored pigments should be mixed with the diluent, if possible, before adding it to the casting material as recommended for silicone rubber (Smith & Rhoton, 1999). Fluorescent agents can be added to PU4ii, which already possess inherent fluorescent properties, enabling the use of confocal microscopy (Meyer et al., 2007). Whitening of casts can be achieved by treatment with 10% hydrogen peroxide as demonstrated for canine silicon tracheobronchial casts (Cope, 2008).

UNUSUAL CASTING MEDIA AND METHODS

India Ink and Gelatin

India ink may be combined with gelatin (Kolts et al., 1994; Ninomiya et al., 2011). This can be achieved by mixing 300 mL of 5% gelatin and 0.5% (v/v) India ink solution and injecting into arteries (Kolts et al., 1994).

Lead Oxide and Milk Mixture

A mixture of lead oxide and milk was used to demonstrate the arterial supply of the long head of biceps brachii tendon in human cadavers (Cheng et al., 2010). This unusual combination had previously been demonstrated to deliver consistently high-quality radiographs largely at par with those from fresh cadavers (An et al., 2012). Prior to injection with lead oxide, the upper limb specimens were injected with 50 mL of 6% hydrogen peroxide via the axillary artery and then submerged in a warm water bath at ~50 °C for 3 h. Preferably, injections are performed in a pulsatile fashion, with the plunger being rhythmically pushed and released by

hand to mimic the normal heartbeat. This technique decreased the risk of vessel rupture (Rees & Taylor, 1986).

Casting Combined with Plastination

Casting may be combined together with plastination to improve quality and fortitude of a specimen. Casting of the vitreous chamber of the porcine eye was achieved with silicone S10 followed by plastination of the wall of the eyeball (bulbus oculi) with PEG (Sivrev et al., 1997). The combination of two chemically different polymers resulted in soft and life-like eyes, safe for student handling and use during anatomical study and also for practicing delicate surgical procedures (Sivrev et al., 1997).

Casting Combined with Histology

Small fragments of tissue injected with PU4ii may be processed by fixation in 2.5% glutaraldehyde, dehydration in ascending concentrations of ethanol and embedded in paraffin wax (Meyer et al., 2007). Sections of 8–10 μm were stained with Haemalaun-Eosin and van Gieson and evaluated for the presence of PU4ii in small vessels (Meyer et al., 2007). PU4ii casting has also been combined with fluorescence microscopy to view thioflavin S-labeled Alzheimer plaques in PU4ii casted brain tissue. Inherent fluorescence of the casting material allows visualizing the relation of the vasculature and plaques simultaneously (Meyer et al., 2008).

Vinyl Acetate Casting

Vinyl acetate is an organic compound with the formula $\text{CH}_3\text{COOCH}=\text{CH}_2$. A colorless liquid with a pungent odor, it is the precursor to polyvinyl acetate. It can be polymerized, either by itself to make polyvinyl acetate, or with other monomers to prepare copolymers such as ethylene-vinyl acetate. About 80–100 mL of solution prepared with 30% acetone colored with red nitrocellulose lacquer was injected into femoral arteries of seven human cadaver specimens (three right side and four left side) (Tanaka et al., 2006). The portion with the femoral artery was dissected out. Specimens were kept without traction, at 15 °C, for a period of 3 days and afterwards taken to corrosion in a 30% sulfuric acid solution for 10 days. After this period, the solution was substituted with a 50% sulfuric acid solution, for another 10 days, after which the specimens were carefully washed in water for 4–5 h (Tanaka et al., 2006).

CONCLUSION

The literature describing casting methods, materials and their uses is vast and progressive. The production of successful casts depends on a number of factors. It starts with the choice of the biological specimen and the appropriate casting material without losing focus of the targeted use and application of the cast. Moreover, those studies pointing out the advantages and disadvantages and/or failure rates are very useful but the number of such studies is quite limited. It is important to point out that the specimens to be

injected comprise biological material, which often makes the outcome unpredictable. It is common that many attempts may be required before obtaining usable casts. The importance of casts in teaching and research cannot be overemphasized. While computer-based learning resources have a complementary role to traditional learning methods, they appear to have significant disadvantages compared to traditional use of specimens in learning nominal anatomy (Khot et al., 2013). Virtual reality shows no advantage over static presentation of key views (Khot et al., 2013) and physical models have a significant advantage over alternative learning resources in enhancing understanding of complex 3D anatomical architecture (Preece et al., 2013). Furthermore, discoveries of novel biological processes such as intussusceptive angiogenesis were achieved through casting (Caduff et al., 1986; Makanya et al., 2009).

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