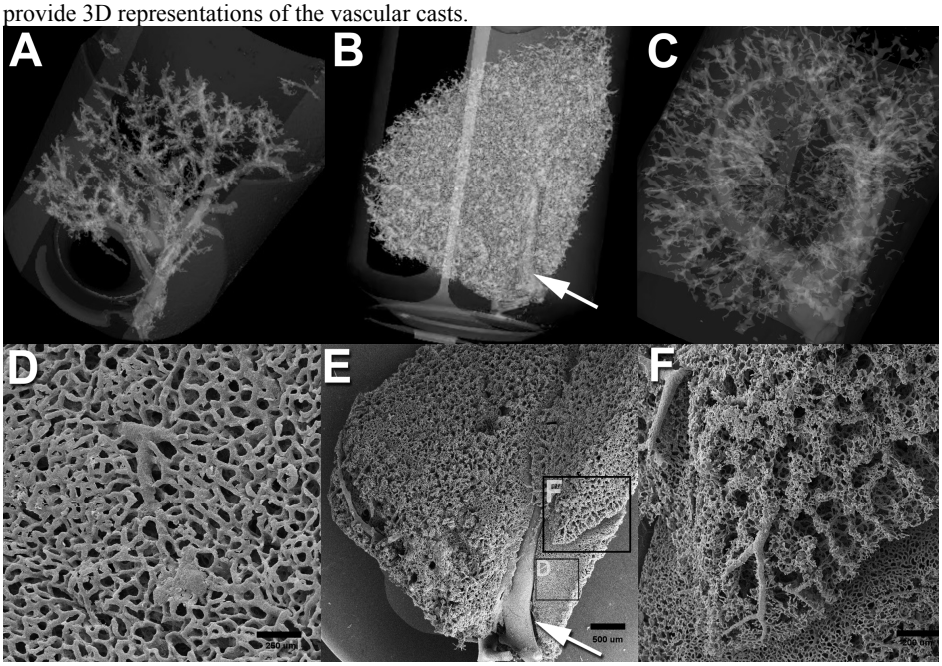
VISUALIZATION OF VASCULAR CASTS USING 3D MR IMAGING

I. Rowland[1], J. Heintz[2], D. Steeber[3], and R. Albrecht[2]
[1]Department of Radiology, University of Wisconsin, Madison, Wisconsin, United States, [2]Department of Animal Sciences, University of Wisconsin, Madison, Wisconsin, United States, [3]Department of Biological Sciences, University of Wisconsin, Milwaukee, Wisconsin, United States

Introduction: Corrosion casting using methylmethacrylate is an established technique for the production of structures representing the 3D intraluminal structure of tissue vasculature [1]. High levels of detail down to the subcellular level may be readily obtained using techniques such as scanning electron microscopy (SEM). In this study, we have utilized MR imaging to obtain images from vascular casts prepared from a variety of organs including liver, kidney and lung. Whilst MR is not able to provide the same level of resolution as SEM, it can provide 3D data that can be compared directly with in vivo MR angiograms. The 3D images of whole organ vasculature can also be used to direct high resolution SEM examination of specific areas of interest. Furthermore, casts with known dimensions, as measured using SEM, could be used to validate direct and indirect MR methods of determining vascular dimensions [2]. Additionally, paramagnetic complexes could be incorporated into the casting material to provide phantoms to represent first pass bolus passage of contrast agent. Previously, attempts to use silicones as casting materials have provided casts that can be imaged directly [3]. Here, however, we have immersed the methylmethacrylate casts in an aqueous solution of MR contrast agent and imaged the water, using the absence of signal to provide 3D representations of the casting material.

Materials and Methods: All studies and procedures were approved by the University of Wisconsin, Madison, Research Animal Resource Center Animal Care and Use Committee. **Vascular Corrosion Casting:** After perfusion-fixation with 1% glutaraldehyde and 3% formaldehyde in PBS, mice were perfused with methyl methacrylate (MMA) resin. Vascular corrosion casting was performed as previously described [4]. Briefly, five mL of MMA (Ladd, Burlington,VT) stabilized with 25 ppm of hydroquinone was mixed with 0.8 grams of benzoyl peroxide, (Dainippon Ink and Chemicals, Tokyo, Japan). Mercox CL-2B (Dainippon Ink and Chemicals) was diluted with an equal volume of this MMA with benzoyl peroxide solution. This mixture was mixed by inversion immediately before use and injected into the perfused mouse. The viscosity of this resin mixture is close to the viscosity of blood, approximately 5 centipoise, allowing for more complete casting of the capillary beds. The methacrylate mixture was allowed to polymerize and tempered overnight at 60^{o}C to ensure complete polymerization and increase the thermal stability of the casts. Tissue was removed by corrosion in 5% potassium hydroxide at 45^{o}C for several days [5]. After several changes in hydroxide the casts were rinsed in H_2O, 100% EtOH (2 x 15 min) and air dried from 100% EtOH. **Scanning Electron Microscopy:** Casts were mounted on aluminum stubs, sputter coated with 10 nm of gold and viewed with an Hitachi S-570 scanning electron microscope at15 KV accelerating voltage. In order to correlate MR data with SEM data, selected areas of the casts were imaged by SEM using the whole organ 3D MR images as a guide. **MR Imaging:** All images were obtained using a Varian Inova small animal imaging and spectroscopy system utilizing a 3cm diameter Varian quadrature volume coil. A 3D gradient echo sequence was used to obtain data from the cast immersed in contrast agent solution (TR=20 ms, TE=6.5 ms, FL=65.0°, Ma=512x256x256, FOV= 24x12x12mm, NEX=32). Images were acquired with an isotropic resolution of approximately 47um. The concentration of contrast agent was optimized for the sequence (5mM MultiHance). The data was converted to Analyze format using ImageJ and then surface rendered using Osirix to provide 3D representations of the vascular casts.



Results and Discussion: Figure 1: Upper row: 3D rendered MR images of (A) liver, (B), lung and (C) kidney corrosion casts obtained with 47 um isotropic resolution. **Lower row:** SEM images (D, E & F) of the same lung sample as shown in B. A branch of the pulmonary artery, is conspicuous at the lower aspect of the lung (B) and (E) (arrowed) Directly to the right of the large vessel is a portion of a lung lobe showing a cast of the capillary network that supplies the pleura covering the surface of the lung lobe (D). The capillary organization is planar consistent with the thin membrane covering the actual lung tissue. Larger feeder vessels can be seen to branch into the net-like capillary bed. A portion of a fractured surface of the lung tissue. The fracture has exposed the internal lung vascular organization. In greater magnification (F) the holes (bronchioles) are interspersed with the capillary network that surrounds numerous alveoli, cup shaped vascular structures. A feeder arteriole can be seen to emerge from the tissue at the lower end of the lobe.

As clearly seen from the Figure, MR data from the (A) liver, (B) lung (C) kidney casts provided excellent 3D representations of the casts that, as shown for the lung specimen, could be compared directly with SEM data. Whilst MR imaging cannot match the resolution readily achievable using SEM, 3D MR data can direct the SEM examination to areas of interest and non-destructively provide complementary information.

References: [1] Scanning Electron Microscopy of Vascular Casts: Methods and Applications (Electron Microscopy in Biology and Medicine, Vol. 10) Eds: Hisao Fujita, H, Motta, PM, Murakami, T, Kluwer Academic Press (1992), [2] Dennie J, Mandeville JB, Boxerman JL, Packard SD, Rosen BR, Weisskoff RM Magn Reson Med 40:793 (1998)**,** [3] Rowland IJ, Vejby Søgaard L, Simonsen, HJ Proc. Intl. Soc. Mag. Reson. Med. 11:1357 (2003), [4] Steeber, D, & Albrecht, RM, Microsc. Res. Tech. 27:353 (1994), [5] Sims, PA & Albrecht, R.M. Scanning. Microsc. 7:637 (1993)