

## Methods to Prepare Perfusion Fixed Cardiac Specimens for Multimodal Imaging: The Use of Formalin and Agar Gels

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Recent advances in cardiac imaging have resulted in a growing understanding of both the form and function of the heart *in vivo*. Currently, the primary modalities for cardiac imaging are (1) two-dimensional cardiac ultrasound or echocardiography, (2) computed tomography (CT), and (3) magnetic resonance imaging (MRI). Yet, high resolution imaging with these modalities can be complicated by motion artifacts and long acquisition times resulting in most of the high resolution anatomical cardiac imaging protocols being reserved for *ex vivo* studies. Our laboratory has

had the privilege to obtain fresh human heart specimens for educational and research purposes. These specimens have been perfusion fixed in 10% buffered formalin, by attaching cannulas to the great vessels, so to create a pressure head of approximately 50 mm Hg. The hearts were then suspended in containers and positioned in anatomically correct orientations before being embedded in 0.7% agar gel, at approximately 45 °C. The cooled specimens were then scanned using the aforementioned clinical imaging modalities (2D and 3D echocardiography, CT, and 3T MRI). The stability of the embedded specimen, the physical properties of the gel, and the lack of motion artifacts allows for the acquisition of extremely high resolution images. These images have subsequently been used in the analysis of cardiac anatomies for a variety of pathologic investigations, not possible with current clinical imaging protocols, and/or for high resolution diffusion tensor MR imaging studies (e.g., of fiber orientations in heart failure in swine ventricles). Future work will include investigations as to whether this gelling approach could be used to prepare other organ specimens for such imaging.

## Design of a Novel Experimental Setup for the Assessment of the Fossa Ovalis Within Large Mammalian Hearts: Investigating Tissue Properties and Clinical Devices Used for Transseptal Access

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Clinical access to the left atrium and/or to the left ventricle for medical device delivery, from a nonsurgical standpoint, continues to be a challenge. Currently such procedures involve left heart access via delivery through the arterial system via the aorta or across the atrial septal wall. More specifically, medical devices delivered through the atrial septum require transseptal punctures by utilizing tools and delivery systems, which include puncturing needles, stiff wires, or more sophisticated approaches, such as wire tips using rf energy. Typically, from the right atrium, one hopes to make this approach through the fossa ovalis and thus gain access to the left atrium. Next, a dilator and an outer device delivery sheath/catheter (clinically available between 7 °F and 24 °F) are employed to pass through the initial puncture and then create a larger hole through the septum. With continued advancements in intracardiac device technologies, it is foreseeable that larger and larger tools may be needed to perform more complicated procedures from such a percutaneous approach (e.g., the

transcatheter deliveries of mitral and/or aortic valves). One of the primary aims of the present study was to assess the relative properties of the fossa ovalis within a large sample of large mammalian hearts and/or the relative amount of forces needed to induce anatomical impacts (i.e., tenting, puncturing, and dilating the fossa ovalis). To do so, an experimental platform has been uniquely developed and experiment data collected. Briefly, the interatrial septums from large mammalian hearts were excised and placed on a plate lined with a silicone elastomer. This plate contains a hole where the fossa is centered and then pinned onto the gel. A smaller plate covers the tissue and is also pinned to the plate, preventing the pins from bending or leaning inward as the tissue is being tested. The prepared sample can then be depressed with a rod attached to a force transducer to predetermined distances and cause strain on the tissue: Resulting forces are digitally recorded. Such experimental protocols can be performed multiple times and to date have allowed for consistent measurements of each fossa ovalis tested. This experimental approach has also allowed us to determine the amount of force required to perforate a given fossa ovalis and thus has provided us with insights relative to device designs. Further use of this setup could be employed to study the transseptal passage and imposed forces on larger transseptal devices; e.g., to determine their potential impacts on the native anatomy of the heart. This novel approach to examine the anatomical and physical properties of the cardiac fossa ovalis should be a great value for those designing or clinically deploying transseptal therapies.