

The E12 Technique as an Accessory Tool for the Study of Myocardial Fiber Structure Analysis in MRI

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Abstract

This paper documents use of the plastination E12 technique to analyze myocardial fiber arrangement and compare its pattern of distribution to magnetic resonance (MR) images. Human hearts were embedded in a "plastic block" consisting of gelatin and polyethylene glycol and scanned using a General Electric Superconducting Magnet (Signa). After scanning the hearts were sectioned and processed for plastination. The E12 plastinated heart sections allowed visualization of the 3-dimensional details of the heart, vessels and myocardial bundles for comparison with the MR images. The myocardial fibers seen in the MR images showed similar gradient directions and details to the anatomical heart sections.

Introduction

The timing and adequacy of reperfusion of an infarcted-related artery (Croisille et al., 1999) determine patient prognosis in acute myocardial infarction. To limit ischemic myocardial injury, it is important to differentiate viable from infarcted (non viable) myocardium. Assessment of viability is based on either stress echocardiography or trace techniques; however, those methods have limited spatial resolution. For a review on predicting myocardial viability by MRI see Higgins (1999).

Magnetic Resonance Imaging (MRI) has the ability to quantify myocardial 3D deformation and strain precisely and non-invasively. In order to document that the MR (magnetic resonance) permit a true comparison between the generated images and the anatomy of the scanned organ, hearts were scanned and then sectioned so that anatomical details of each slice could be compared to the details in the image. The use of plastinated specimens seemed ideal for this purpose. The acquired transparency of the specimens as final result of the E12 technique permitted a 3D analysis and true comparison to the images generated by the MRI. Plastinated specimens can be stored for long-term use, and do not give off fixative fumes. Plastination is also a relatively simple and easier method to obtain clear specimens when compared to traditional methods such as Spalholz (1924) and Tompsett (1956).

Since the birth of plastination in the mid-80s, several authors have successfully used plastinated specimens to correlate morphology with MRI in research, education and clinical medicine (McNiesh and von Hagens, 1988; Baptista et al., 1990; Ripani et al., 1993, 1996; Hussain et al., 1996; Magiros et al., 1996, 1997; Cook, 1997; Entius et al., 1997).

This paper introduces the E-12 technique as a tool for the comparative analysis of myocardial fiber distribution study using MRI.

Materials and Methods

Materials

Several human hearts were used for this study. The hearts were removed from cadavers and washed in tap water to remove blood and blood clots from the chambers, coronary arteries and cardiac veins. After the blood was removed, the heart chambers were filled in with cotton to maintain the cavities in the dilated state.

The coronary arteries were injected with a mixture of 30% gelatin and 0.1ml (0.05mmol)/kg of gadopentetate dimeglumine (Magnevist® - Berlex Laboratories, Inc., Wayne, NJ 07470, USA). Eosin (Sigma-Aldrich, 3050 Spruce Street, St Louis, MO 53178, USA) was added to the mixture for color.

The hearts were then immersed in a 10% formalin solution for 24 hours. After fixation the hearts were prepared for MRI scanning.

Preparation of the heart for scanning

The hearts were immersed in a solution of gelatin (15 gm/100ml) and polyethylene glycol 1000 (10ml/100ml of gelatin solution) using the method described by McCormick (1961). This plastic/gelatin solution was transparent and after solidifying maintained the position of the heart during the MRI scan and during physical sectioning.

Scanning

MRI images were obtained using the General Electric Superconducting Magnet (Signa) operating at 1.5 Tesla with a corresponding resonance frequency for protons of 63.9 MHz.

The scan protocol is a balanced matrix spin echo gated acquisition, (TR=2000 msec, TE=20 and 60 msec) for oblique, sagittal and/or coronal short and long axis views of the myocardium. Two images were generated at each anatomic level: one was formed from the first spin-echo (TE=20 msec) and the other from the second echo (TE=60 msec). The MR scan slice thickness was 3.0 mm with a skip distance of 3.0 mm. The images were archived to magnetic tape and transferred to the imaging workstation for analysis. Medical College of Ohio (MCO) software on the DEC microVax workstation allowed display, image processing and texture analysis of the images.

Image processing and mathematical codes have been written on the DEC microVax Workstation to analyze texture on the intensity distributions of the tissues scanned in the MR scanner. The cardiac slices were preserved by plastinau'on for future study and permanent storage.

Sectioning

The "plastic block" (gelatin and polyethylene glycol) was sectioned using an electric circular meat slicer. Three millimeters (3 mm) sections were made parallel to the long axis of the left ventricle. Each slice was placed between plastic grids and immersed in cold acetone (-25°C) for dehydration.

Dehydration

Freeze substitution as described by von Hagens (1985) and von Hagens et al. (1987) was used for dehydration. Three changes of acetone were necessary, with each bath containing a volume of acetone 5-10 times the volume of the specimens.

After dehydration, the heart sections were immersed in methylene chloride for defatting. Two weekly changes of methylene chloride were necessary. After defatting the sections were impregnated with epoxy.

Impregnation

Impregnation using an epoxy based reaction mixture (E12) technique was performed at room temperature. The epoxy reaction mixture was: E12/AT30/AT10/E1 (95:5:20:26 pbw).

The specimens were submerged in the reaction mixture and placed in a vacuum chamber, directly from the methylene chloride solution. Pressure was reduced rapidly to 10mmHg. The rapid boiling out of the methylene chloride caused the temperature of the mixture to be reduced drastically. This helped to control the exothermic reaction taking place (Weber and Henry, 1993). Impregnation was accomplished in 24 hours.

Casting

The heart sections were cast between two sheets of tempered glass and a flexible gasket (Parker O-Ring - Zatkoff Seals & Packing, 8929 Airport HWY. Holland, Ohio 43528-9604, USA) was used as spacer. The following mixture was used as the casting resin: E12/AT30/E1 (95:5:26 pbw).

The specimens were placed between two glass plates, sealed and the molds filled with the casting mixture. After the molds were filled in they were placed inside a vacuum chamber to remove small bubbles present in the resin. This took place in 45 minutes to 1 hour. Larger bubbles were afterwards removed manually. After bubble removal, the mold was placed at horizontal 15° incline to assure correct positioning of the heart slice in the mold. When the polymer showed more viscosity and was tacky (2 to 3 days) the specimens were placed in an oven at 40°C for 10 days. Hardening was completed when "Newton" rings were seen in the casting molds. The glass plates were removed carefully and the sheets were cut and trimmed as desired.

Cutting and sanding the molds

A bend saw (Sears Roebuck and Co., 3333 Beverly Road, Hoffman Estate, IL 60179, USA) was used to cut and trim the plastic along the edges of the heart slices. Belt and disc sanders from Sears Roebuck and Co. were used to remove sharp edges from the plastic slices.

Photographic images of the 3.0 mm anatomical sections and MR scan slices were compared.

Results

In order to evaluate the quality of cardiac imaging of the GE MR scanner at the Medical College of Ohio, and to verify anatomical details on the MR images, several cadaver hearts were imaged and sectioned at 3 mm intervals. The heart images were processed using zoom, filters contour and cut modules. The maximum gradient images were then obtained and filtered. The gradient images were subtracted from the original images of the myocardium using an absolute subtraction method, to enhance the direction and structure of the muscle bundles in the myocardium. The heart sections processed by the E12 technique were correlated with the MR images showing that the myocardium can be identified in the computer-processed images. Maximum gradient processing emphasizes the direction of the muscle bundles and such images show similar gradient directions and details seen with the anatomical heart sections. Examples of the resulting anatomical slices and an intermediate scanned image are shown in figure 1.

Discussion

Magnevist® (that was injected in the coronary arteries mixed with gelatin and red dye) is a paramagnetic metal ion chelate used in clinical diagnostic procedures as an injectable enhancement agent to enhance signal intensity, and thus visual contrast, in magnetic resonance imaging.

McCormick's method (1961) to obtain cross sections of

fresh tissue was helpful to align and section the heart similar to the coordinates used for MR scanning. Positioning the heart in the mixture of gelatin and polyethylene glycol 1000 ensured stability during the scan as well as during sectioning. Marking the MR scan lines on the container facilitated sawing the specimen in the same planes as the scans. The resultant images were closely related to the scan sections.

Parker O-Ring from Zatkoff was an ideal locally available product to use for the gasket in the plastination mold. It is of a flexible base polymer, nitrile and is supplied in a variety of diameters (3 mm to 10 mm). In our case the thickness used was 3 mm.

The E12 sheet specimens allowed visualization of the 3-dimensional detail of the heart, vessels and myocardial bundles for comparison with the MR images. Maximum gradient processing emphasizes the direction of the muscle bundles and such images show similar gradient directions and details with the anatomical heart sections.

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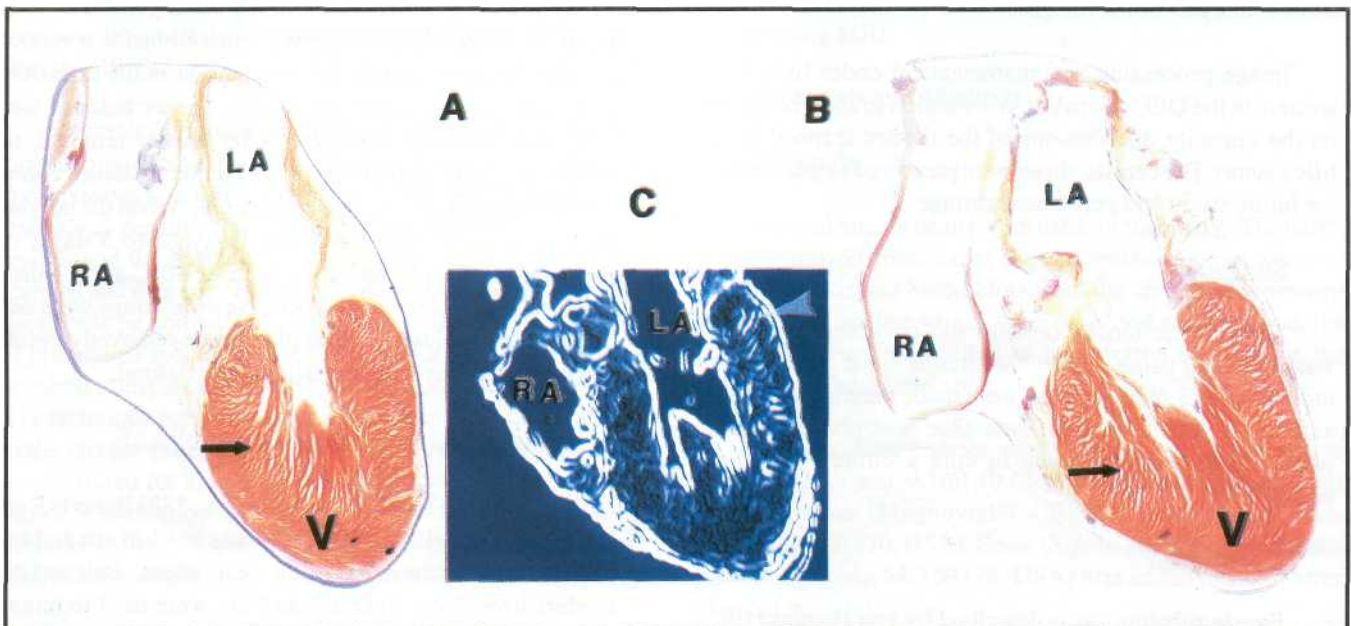


Figure 1: Photographs of plastinated heart sections (A, B) and an intermediate magnetic resonance image (C) showing the following structures: left atrium (LA); right atrium (RA); left ventricle (V) and myocardial bundles as fibers (arrows).

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