Whole heart histology a method for the direct integration of histology with electrophysiological and imaging data

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Letters

RESEARCH CORRESPONDENCE

Whole Heart Histology

A Method for the Direct Integration of Histology With Electrophysiological and Imaging Data

Although recent advances have been made in the delineation of ventricular tachycardia substrate using various imaging modalities and mapping approaches, these methods are rarely validated by histology, which remains the true gold standard (1). When histological validation has been performed, a side-by-side approach is usually used, which results in a significant margin of error. A significant factor hampering the accurate comparison of different modalities is the deformation and shrinkage/contraction of the heart, which occurs as soon as the chambers are evacuated of blood. Here, we present a method of histological validation of both electrophysiological and imaging data with a high degree of accuracy that is both easy to use and adapt to various settings and modalities (2,3).

After death, or in the case of animal models, sacrifice, the heart is excised and rinsed (**Figure 1A**). The chambers are filled with the water-dissolvable cold polymer HistOmer (HistOtech, Aarhus, Denmark) (4) to maintain chamber dimensions (**Figure 1B**). The heart is fixed with formaldehyde by coronary perfusion followed by submersion in formaldehyde. After full fixation is achieved, the heart is placed in an magnetic resonance imaging–compatible polyvinyl chloride box, and the box is filled with HistOmer (**Figure 1C**). The block of HistOmer containing the heart is removed from the box and placed in the HistOtech Quick Slicer Flex (HistOtech) and sliced into 4-mm-thick short-axis slices with the HistOtech Pathology Knife (HistOtech) (**Figure 1D**). Each slice is photographed before the HistOmer is peeled off the tissue, and the slices are stored in alcohol-glycerin (**Figures 1E and 1F**). Because HistOmer is MRI compatible, ex vivo imaging can be performed at any point in the process. **Figure 1G** shows one approach: in a swine model, after mapping, intravenous gadolinium is administered 20 min before sacrifice. The hearts are then imaged after filling with HistOmer but before formaldehyde fixation. Alternatively, imaging can be performed after fixation but before slicing.

The photographs taken of the gross pathological slices are processed as a DICOM file and imported into MASS, Research version 2016 (Leiden University Medical Center, Leiden, the Netherlands), where the cardiac contours can be traced and exported as .vtk files. These .vtk files are then imported into CARTO (Biosense Webster, Irvine, California) and merged with the in vivo mapping data (**Figure 1H**). In our swine model, 3 ablation lesions are placed in the left ventricle endocardium during mapping, and these 3 lesions are used as landmarks. Alternatively, anatomic landmarks may be used. Once merged, the data are exported and visualized in either MASS or Paraview, version 5.6 (Kitware Inc., Clifton Park, New York). In vivo and/or ex vivo imaging data can also be imported and visualized in this environment.

Locations on the gross pathological slices corresponding to either electrophysiological data points or to imaging locations of interest can be excised, embedded in histological cassettes, and processed in a standard histology laboratory. Multiple cassettes can be used to process areas larger than 1 standard cassette.

In its essence, this approach is simple to apply and is easily reproduced in different settings. Filling the chambers results in the preservation of cardiac dimension, and use of landmarks to merge 3-dimensional structures removes the large registration error resulting from side-by-side comparison of arbitrary slices. Furthermore, because this method results in zero pathological waste, multiple histological approaches may be applied, including multiple (immunofluorescent) stainings and 3-dimensional microscopic reconstructions.

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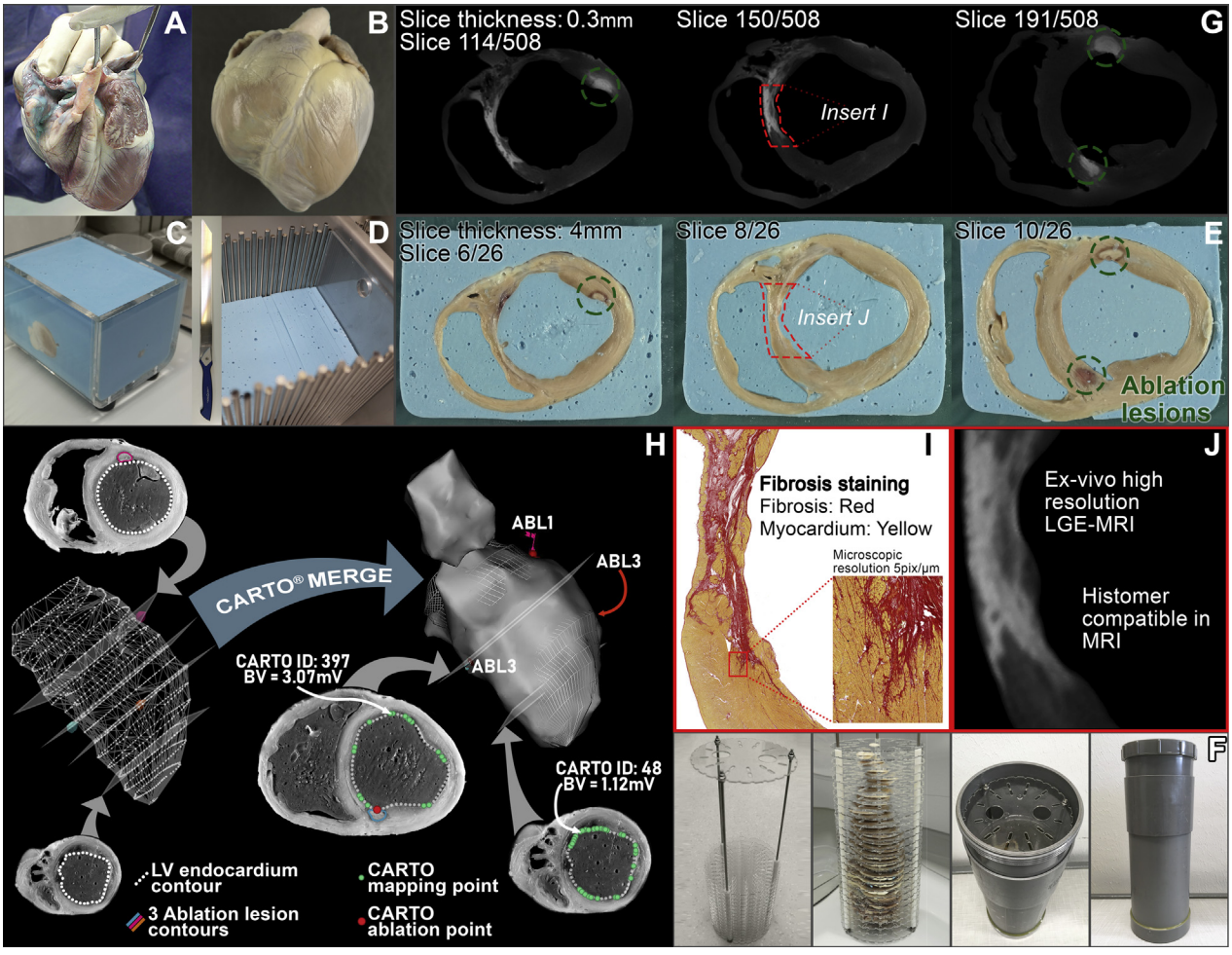
FIGURE 1 Method for Direct Integration of Histology With Electrophysiological and Imaging Data



(A) Intact heart is rinsed before being filled with HistOmer (blue material visible). **(B)** Once filled, to maintain chamber dimensions, the heart is fixed in formaldehyde. **(C)** The fixed heart is place in a polyvinyl chloride box, which is filled with HistOmer. **(D)** The HistOmer block can be removed and placed in the HistOtech Quick Slicer Flex and sliced into 4-mm-thick gross pathological slices using the HistOtech Pathology Knife. **(E)** Gross slices are photographed. Ablation lesions are clearly visible on gross pathology. **(F)** Sliced heart is stored in alcohol-glycerin in custom-made storage units. **(G)** Ex vivo 0.3-mm isotropic late gadolinium enhanced magnetic resonance imaging (gadolinium administered shortly before animal sacrifice) is aligned with gross pathological slices. **(H)** Contours are traced onto the images of the gross pathological slices in MASS, Research version 2016 (Leiden, the Netherlands), and the resulting .vtk files are imported into CARTO, where landmarks (in this case, 3 ablation [ABL] lesions) are used to merge the ex vivo shell with the in vivo map. Once merged, the data are exported and visualized (in MASS or Paraview). The mapping data are projected onto the gross pathological slices, and histological samples can be taken at precise locations. **(I)** The region of interest is processed histologically (here, stained with Sirius red for fibrosis). **(J)** The corresponding area on magnetic resonance imaging.

Please note: The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Clinical Electrophysiology* author instructions page.

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