Mapping the human pulmonary venoatrial junction with optical coherence tomography

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Abstract: Imaging guidance provided by optical coherence tomography (OCT) could improve the outcomes of atrial fibrillation (AF) ablation by providing detailed structural information of the pulmonary veins, which are critical targets during ablation. In this study, stitched volumetric OCT images of venoatrial junctions from post-mortem human hearts were acquired and compared to histology. Image features corresponding to venous media and myocardial sleeves, as well as fiber orientation and fibrosis, were identified and found to vary between veins. Imaging of detailed tissue architecture could improve understanding of the AF structural substrate within the pulmonary veins and assist the guidance of ablation procedures.

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1. Introduction

Understanding the myocardial structure of the pulmonary veins (PVs) has become of significant importance since the discovery that atrial fibrillation (AF) can be initiated by ectopic beats originating within the PV myocardial sleeves [1]. Histological studies have shown that the myocardial sleeves of the PVs may extend as far as 25 mm into the vein [2,3] and can have complex myofiber arrangement with crossing and mesh-like patterns [2,4]. The intricate structure of the PVs have been linked to AF dynamics, with studies in canines showing regions of myofiber complexity correlated to electrical conduction disturbances [5,6], while arrhythmogenic high-frequency potentials have been associated with thickened PV walls [7]. AF patients have also been reported to have higher degrees of discontinuity, hypertrophy, and fibrosis within their PV myocardial sleeves [3]. These studies emphasize the complex and variable PV tissue structure and that these structural features may have a significant influence on AF mechanisms.

Detailed imaging of PV tissue structure could improve the understanding of the structural substrate underlying AF as well as provide guidance to improve ablation, which still suffers from variable success [8]. The current knowledge of PV tissue structure has most commonly been acquired through histological studies, but histology is destructive and cannot be applied in vivo for ablation guidance. High frequency intravascular ultrasound has been used to image the PVs in vivo and is able to capture the thicknesses of the different layers of the vein wall, as well as the lengths of the myocardial sleeves [7,9]. However, the ability of ultrasound to resolve finer features such as fibrosis and fiber orientation in detail may be limited.

Optical coherence tomography (OCT) is an optical imaging modality capable of capturing 3D image volumes with micrometer-scale resolution and millimeter-scale imaging depth. Due to its high resolution and non-destructive imaging capability, OCT presents an attractive option for imaging cardiac tissue. OCT is capable of distinguishing specific cardiac tissue compositions and has been used to classify endocardium, adipose tissue, and myocardium in human atria [10]. Additionally, imaging with OCT-integrated catheters have been carried out in vivo in swine [11,12], demonstrating the potential to use OCT for real-time, ablation guidance. OCT imaging during PV stenosis has also been used to image fibrous plaque, microvessels, and thrombi [13], and the imaging of pulmonary venoatrial junctions using
OCT has recently been shown to provide information on muscular bundle arrangement in sheep PVs [14]. Therefore, OCT offers promising potential to provide high-resolution imaging of PV structure and in vivo ablation guidance.

The ability of OCT to identify the architecture of the venoatrial junctions in human tissue, however, has not yet been comprehensively investigated. Thus, the objective of this study was to assess the capability of OCT to image important structural features at the human venoatrial junction, specifically fiber orientation, fibrosis, and the presence of myocardial sleeves. Volumetric images of the venoatrial junctions from post-mortem human hearts were acquired with OCT, ex vivo, and the images were compared to histology.

2. Methods

2.1 Tissue acquisition

Diseased human hearts (n = 10) were acquired under an approved protocol from the National Disease Research Interchange. The inclusion criteria for the protocol was based on the following diagnoses: end stage heart failure, cardiomyopathy, coronary heart disease, amyloid, AF, and myocardial infarction. All specimens were de-identified and were exempt from human subjects research study, according to the Department of Health and Human Services human subject regulation exemption 4. Donor characteristics are provided in Table 1. The average age was 63.2 years. One donor had a history of AF, four had congestive heart failure, two had myocardial infarction, and two had valvular heart disease. Recovery of hearts was completed within 24 hours after death, and the hearts were delivered to the lab while submerged in an ice-cold phosphate-buffered saline bath. From the 10 hearts, 32 pulmonary venoatrial junctions were imaged.

| Heart # | Age | Sex | Cardiovascular Disease History |
|---------|-----|-----|-------------------------------|
| 1       | 77  | F   | CAD, HTN, CHF, AF, PVD        |
| 2       | 70  | F   | CHF, HTN                      |
| 3       | 46  | F   | HTN, CAD, MI                  |
| 4       | 67  | M   | MI, HTN, HLD                  |
| 5       | 59  | F   | HTN                           |
| 6       | 67  | M   | CHF, VHD, HLD                 |
| 7       | 58  | M   | CAD, CHF, HTN, HLD            |
| 8       | 68  | M   | HTN, CAD, HLD                 |
| 9       | 62  | F   | HLD, CAD                      |
| 10      | 58  | F   | VHD                           |

AF: atrial fibrillation; CAD: coronary artery disease; CHF: congestive heart failure; HLD: hyperlipidemia; HTN: hypertension; MI: myocardial infarction; PVD: peripheral vascular disease; VHD: valvular heart disease

2.2 Tissue dissection

Upon arrival to the lab, the left atrium (LA) and PVs were isolated from the rest of the heart. Afterwards, the LA and PVs were dissected to flatten the tissues for imaging with OCT. An example of the dissection procedure is depicted in Fig. 1. The LA was halved, separating the left PVs from the right PVs as shown by the dotted black line in the left-most panel of Fig. 1. Afterwards a longitudinal cut was made along the side of each vein and into the LA, as represented by the dotted red line in Fig. 1. This dissection opened up the vein while keeping the venoatrial junction intact. One vein in Heart #3 did not undergo this dissection because the LA had not been fully intact upon arrival to the lab and was already sufficiently flat for imaging. The flattened tissues were pinned to a corkboard and submerged in a 10:1 phosphate buffered saline solution.
2.3 OCT imaging protocol

All tissues were imaged fresh with no prior fixation. The tissues were imaged with the TELESTO I (Thorlabs GmbH, Dachau, Germany) spectral-domain OCT system, which has an axial resolution of 6.5 μm, lateral resolution of 15 μm, and imaging depth of 2.51 mm in air. The system uses a matched pair broadband superluminescent diode light source, with a center wavelength of 1325 nm and a bandwidth of over 150 nm. It uses a linear InGaAs array-based spectrometer. Its maximum axial line rate is 91 kHz, and imaging was carried out at 28 kHz. The OCT-IMM3 immersion-style sample z-Spacer (Thorlabs GmbH, Dachau, Germany) was used to image the tissues while they were submerged in a phosphate buffered saline solution. Overlapping, 3D image volumes were obtained to cover the pulmonary venoatrial junctions. Each individual image volume had dimensions of 800 x 800 x 512 voxels corresponding to 5.00 x 5.00 x 1.79 mm, using a refractive index of 1.4 for atrial tissue.

2.4 Histology

After imaging, the tissues of the imaged areas were cut into blocks and placed in 10% formalin for at least 24 hours fixation. The blocks had maximum x-y dimensions of 40 mm by 30 mm, and 5 μm thick levels were obtained through the entirety of each block with a maximum of 3 mm between each level. The levels were obtained in the same orientation as the OCT B-scans, except for select regions in heart #8 where levels in the en face view were also obtained to enable comparisons of fiber orientation between OCT and histology. The tissues were stained with Masson’s Trichrome, and a Leica SCN400 (Leica Microsystems, Wetzlar, Germany) slide scanner with x40 magnification was used to digitize the histology slides. Afterwards, different tissue types were identified from the slides by a cardiovascular pathologist blinded to the OCT images. Histology slides were matched to OCT images acquired from the same location that the histology block had been excised from. Locations of the histology blocks and OCT volumes were determined based on camera images taken during the cutting of the histology blocks and white light images acquired by the TELESTO I system that depicted the field of view within each OCT volume.

2.5 OCT image stitching

The overlapping image volumes were registered and stitched into composite image volumes to better visualize the tissue features. First, the image volumes were manually registered in the en face plane (x and y-dimensions) based on matching features in the white light images and known lateral translations between each volume during imaging. Custom MATLAB software was used to manually align overlapping white light images and determine the lateral offsets between each consecutive, overlapping pair of image volumes.
To ensure the tissue surfaces were axially aligned in the stitched image volume, the tissue surfaces of each volume were flattened to a uniform depth. Surface detection was carried out on each B-scan in every image volume. First, the B-scan was median filtered with a 3 x 3 kernel to smooth speckle noise. Afterwards, the filtered B-scan was thresholded to separate the image background from the tissue regions. The threshold was empirically determined from a subset of B-scans, and the same threshold was applied to all image volumes. The tissue surface and the contact glass from the z-Spacer are both highly reflective, leading to the surface of the z-Spacer being segmented along with the tissue region and interfering with surface detection. Because the z-Spacer surface can be seen as a thin, primarily horizontal straight line within the B-scan, the z-Spacer contact glass signal was removed from the thresholded image by morphological opening using a 15 x 1 rectangular structuring element. With the z-Spacer signal removed, the tissue surface was typically the only region remaining in the segmented image. Therefore, the pixel location of the maximum intensity in each column was taken as the axial position of the tissue surface. The detected tissue surface was smoothed by a 1D median filter with a kernel size of 25. After the location of the tissue surface in a B-scan was determined, the pixels of each column were shifted up the number of pixels necessary to place the tissue surface at a uniform depth throughout the image.

After the lateral offsets between consecutive, overlapping pairs of image volumes were determined and the tissue surfaces of all image volumes were flattened to a uniform depth, the image volumes could be stitched together to form the composite image volume. The placement of each image volume in the stitched domain was determined by solving a linear system of equations formed from the known lateral offsets. The coordinates of the origin for an image volume $i$ can be denoted as $(x_i, y_i)$. The offsets in the x and y dimensions between the origins of consecutive, overlapping image volumes $i$ and $i + 1$ can be denoted as $Dx_{i(i+1)}$ and $Dy_{i(i+1)}$ respectively. Note that the locations of the image volumes along the z-axis did not need to be determined because flattening the tissue surfaces ensured all image volumes were at the same axial location. Given $n$ individual image volumes and selecting a minimum $x_i$ and $y_i$, $2(n-1)$ unknowns needed to be determined to identify each volume’s location in the stitched domain. A set of $2(n-1)$ equations of the forms $x_i - x_i + 1 = Dx_{i(i+1)}$ and $y_i - y_i + 1 = Dy_{i(i+1)}$ could be created from the known lateral offsets, and the system of equations was solved in matrix form. Once the location of each image volume in the stitched domain was determined, stitching was carried out using gain compensation and multiband blending methods as have been described in prior work [15] to smooth the transitions between image volumes. The stitched image volumes were downsampled by three to decrease computational burden and improve ease of visualization. For visualization, a uniform contrast adjustment was applied over the entirety of each b-scan or en face image until the image features were clear.

### 2.6 Endocardial and myocardial measurements

Because visibility of myocardial features in OCT is sensitive to the thickness of overlying connective tissue, measurements of endocardial thickness were acquired to identify the maximum endocardial thickness under which myocardial features could be seen within this OCT system’s imaging depth. Because variations of endocardial thickness occur around a single venoatrial junction, measurements of endocardial thickness were obtained from three points per PV. These points were taken from regions where fibrosis and fiber orientation were visible at the venoatrial junction as determined qualitatively from the en face OCT images. Three measurements were measured manually from OCT b-scans, calculated using a refractive index of 1.4, with another three measurements acquired from corresponding histology images for comparison.

Additionally, different PVs were found to have different distributions of collagen fibers within the myocardium. To provide a quantitative measure of these differences, texture analysis was carried out in the myocardial regions of representative PVs. A 4.0 x 8.6 x 0.1
mm region of interest (ROI) was extracted from the myocardial region of the PVs. Within each b-scan in the 3D ROI, the local range, standard deviation, and entropy were computed with a 3 x 3 kernel, and then these values were averaged over the entire ROI. Fiber orientation angles were also extracted from each en face image over depth in the ROI, using a previously described gradient-based method [16]. The circular standard deviation was calculated from all of the extracted angles in the 3D ROI to quantify variation in fiber orientation. Fiber orientation angle extracted using the gradient-based method was also plotted against depth to quantify transmural trends.

3. Results

OCT imaging was able to distinguish areas of PV myocardial sleeves, as well as show the directionality and density of collagen fibers and myofiber orientation. The identification of these tissue features was dependent on the thickness and density of overlying connective tissue. Additionally, characteristics of the endocardium such as layer thickness and myointimal thickening could be determined.

3.1 OCT features of endocardium and myocardium

The endocardium was characterized by a highly backscattering, homogeneous layer on the tissue surface. When the entire thickness of the endocardium was within the imaging penetration depth, a sharp transition from the highly backscattering upper layer to a lower intensity region underneath could be observed, indicating a transition to myocardial tissue as shown in Fig. 2(a). This distinct boundary between the upper layer of connective tissue and the myocardium underneath also applied to myocardium underneath venous media. Because the venous media and endocardium blend at the venoatrial junction, connective tissues overlying myocardium will hereafter always be referred to as endocardium for simplicity. The boundary between the endocardial and myocardial layers enabled identification of endocardial thickness. However, if the endocardium was thicker than the penetration depth, a gradual fall-off of intensity in the upper layer was observed instead, as shown in Fig. 2(b). Significant myointimal thickening, consisting of a layer of loose collagen and myointimal cells on the tissue surface, could be identified by a second, highly backscattering layer with a well-delineated boundary with the endocardial layer underneath, as shown in Fig. 2(c). Additionally, collagen fibers could be identified by backscattering strands within regions of myocardium, as shown in Fig. 2(d).

![Fig. 2. OCT b-scans (top) and corresponding Trichrome histology (bottom), showing OCT image textures and layer patterns corresponding to different endocardial and myocardial compositions. A, Endocardium with thickness within OCT depth penetration. B, Endocardium with thickness beyond OCT depth penetration. C, Endocardium with myointimal thickening. D, Endocardium with underlying myocardium with collagen fibers. Dotted yellow lines show representative regions of transition between the endocardium and myocardium. Dotted blue lines show representative regions of transition between loose collagen with myointimal cells and the endocardium. Scale bars represent 1 mm. e = endocardium; m = myocardium; i = loose collagen and myointimal cells; asterisk = imaging artifact from the sample zSpacer.](image)

When shown in the en face view, striations within the OCT images could be correlated to fiber orientation seen in corresponding en face histology images, demonstrated in Figs. 3(a)-
3(b). *En face* views revealed myofiber and collagen fibers wrapping around the PVs in different directions, as shown in Figs. 3(c)-3(d). In particular, in Heart #3, transmural changes in myofiber orientation could be clearly observed, shown in Figs. 3(e)-3(f) where the fiber orientation changed by about 60° at different depths. The extracted profile of fiber orientation over depth shown in Fig. 3(g) shows an abrupt change in fiber orientation, which is consistent with prior findings of transmural left atrial myofiber patterns [17].

Fig. 3. *En face* OCT and histology images, showing fibrosis and fiber orientation. A, OCT *en face* image, shown 0.39 mm from the tissue surface, from heart #8. B, Corresponding *en face* histology image. Dotted yellow lines in the OCT image show boundaries between regions of endocardium and myocardium. White arrowheads indicate striations indicative of fiber orientation in OCT. C, OCT *en face* image, shown 0.30 mm from the tissue surface, from heart #3. D, OCT *en face* image, shown 0.38 mm from the tissue surface, from heart #5. E, Region within the dotted red box shown in C, at a depth of 0.29 mm. F, Region within the dotted red box shown in C, at a depth of 0.17 mm. G, Fiber angle over depth at the point indicated by the black asterisk in C. White double-sided arrows indicate general myofiber orientation trends. All scale bars indicate 1 mm.
3.2 OCT features of the venous endothelium, media, and adventitia

In regions of the PVs consisting of only the venous endothelium, media, and adventitia, the OCT images typically showed a more heterogeneous texture and a deeper penetration depth with a gradual fall-off of intensity. The specific texture of the venous tissues could vary between PVs based on the composition and density of fibrous tissues within the region. For example, in Fig. 4(a), the venous media and adventitita have a speckled appearance, while Fig. 4(b) instead shows a highly backscattering upper layer with additional bands underneath. Finally, some PVs consisting of dense connective tissue had a more homogeneous texture and gradual signal fall-off, similar to regions of thick endocardium as shown in Fig. 4(c).

Fig. 4. OCT b-scans (top) and corresponding Trichrome histology (bottom), showing differences in OCT image texture corresponding to venous media and adventitia with different densities and distributions of connective tissue. A, Venous media and adventitia with a speckled image texture in OCT. B, Venous media and adventitia with a layered image texture in OCT. C, Venous media and adventitia with a smooth homogeneous image texture. Scale bars represent 1 mm. v = venous media; asterisk = imaging artifact from the sample z-Spacer.

3.3 OCT features at the venoatrial junction

Given the distinct image features of different tissue compositions as described above, regions of myocardial sleeves could be differentiated from PV regions containing only venous endothelium, media, and adventitia. The transition from myocardium to adventitia, where the myocardial sleeve ends, could typically be identified by at least one of two ways. First, the sharp boundary between the endocardium and myocardium became more diffuse when entering regions containing only venous media and adventitia, often accompanied by an increase in penetration depth. Second, a more heterogeneous image texture was typically seen with venous media and adventitia compared to endocardium and myocardium. Representative examples are described below, showing how the myocardial sleeves, as well as fibrosis and variations in endocardial thickness, could be observed.

In Fig. 5, the distinct layer boundary disappears and the penetration depth increases once entering the region of only venous endothelium, media, and adventitia, as seen towards the right of the B-scan of Fig. 5(b). The en face view in Fig. 5(a) also shows a clear difference in texture and backscattering between the myocardial region on the left and the region without myocardium on the right of the dashed green line. Collagen distributed between myofibers, as seen from histology, were identifiable within OCT by highly backscattering strands, with the parallel directionality of the fibers clearly defined. The 3D ROI from which texture and fiber orientation statistics were extracted is represented in the x-y and x-z planes by the blue boxes in Figs. 5(a) and 5(b). The mean local range in the myocardial ROI was 5.37, the mean local standard deviation was 1.81, and the mean local entropy was 3.07. The circular standard deviation of extracted fiber orientation angles was 17.03°.
In Fig. 6, the region containing only venous endothelium, media, and adventitial tissues has a more heterogeneous texture, with more variations in intensity and an almost hatched pattern as seen in the *en face* view of Fig. 6(a). The change in image texture is also noticeable in the B-scan shown in Fig. 6(b), where the region without myocardium includes additional bands underneath the upper layer. As demonstrated previously in Fig. 4, different PVs had different textures corresponding to the venous endothelium, media, and adventitia, and other PVs had a speckled texture in both the *en face* and b-scan views, instead of the hatched and layered pattern observed in Fig. 6.
Fig. 6. OCT imaging of a venoatrial junction and corresponding histology, showing change in image texture near the end of the myocardial sleeve. A, Stitched en face region, shown 0.50 mm from the tissue surface from heart #4. B, Stitched b-scan corresponding to the orange line in D. C, Corresponding Trichrome histology to E. Dotted white lines show the approximate location of the PV ostia. Dashed green lines show the approximate area of transition from myocardium to venous media and adventitia. Dotted yellow lines show representative regions of transition between the endocardium and myocardium. All scale bars indicate 1 mm. LA = left atrium; LIPV = left inferior pulmonary vein; e = endocardium; m = myocardium; v = venous media.

Another representative case showing distinct features at the venoatrial junction is given in Fig. 7. With OCT imaging, fibrotic myocardium could be identified past the PV ostium and inside the venous region. The endocardial thickness could be seen to abruptly decrease inside the PV area and significant focal thickening of endocardium was observed near the ostium, as seen in both the en face view in Fig. 7(c) and b-scan view in Fig. 7(d). Again, collagen fibers could be identified by bright strands. Within this PV, the collagen fibers varied more greatly in size and directionality compared to those shown in Fig. 5(a). Similar patterns of complex fibrosis were observed in the LSPV and LIPV of heart #14. The 3D ROI from which texture and fiber orientation statistics were extracted is represented in the x-y and x-z planes by the blue boxes in Figs. 7(c) and 7(d). The mean local range in the myocardial ROI was 4.88, the mean local standard deviation was 1.65, and the mean local entropy was 2.89. The circular standard deviation of extracted fiber orientation angles was 35.17°. The depth penetration increases at the far right of the B-scan in Fig. 7(d), signaling the end of the myocardial sleeve and transitioning to a region containing only the endothelium, venous media, and adventitial tissues.
Endocardial thickness and composition of the venous media and adventitia varied between pulmonary veins and among different regions of a single pulmonary vein. There were some cases in which it was difficult to distinguish the myocardial sleeves from venous regions without myocardium. Figure 8 presents two representative cases. In Fig. 8(a), the increase in depth penetration due to myointimal thickening could deceptively be interpreted as a region consisting of only venous endothelium, media, and adventitia despite myocardium being present underneath. In Fig. 8(c), the upper layer boundary becomes more diffuse, but the penetration depth remains similar throughout, making identification of the end of the myocardial sleeve unclear. The ability to differentiate regions of myocardial sleeves to regions without myocardial sleeves, based on qualitative OCT image features, was generally determined for each PV by a single observer not blinded to the histology. Out of 32 pulmonary venoatrial junctions, it was determined the myocardial sleeves could be differentiated from venous regions without myocardial sleeves in 18 cases while 6 cases were ambiguous. In the remaining 8 cases, the PVs had been cut short such that only the
myocardial sleeves could be imaged, with no transition to venous regions without myocardium, and thus were not counted.

Fig. 8. Cases where identification of pulmonary vein sleeves is ambiguous in OCT imaging. A, OCT b-scan near the LIPV from heart #7. B, Corresponding Trichrome histology to C. C, OCT b-scan near the LSPV from heart #4. D, Corresponding Trichrome histology to C. Dotted yellow lines show representative regions of transition between the endocardium and myocardium. Dotted blue lines show representative regions of transition between loose collagen with myointimal cells and the endocardium. Dashed green lines show the approximate area of transition from myocardium to transmural connective tissue. All scale bars indicate 1 mm. e = endocardium; m = myocardium; i = loose collagen and myointimal cells.

3.4 Endocardial thickness measurements

The ability to visualize myofiber orientation and fibrosis with OCT is also dependent on the thickness of overlying connective tissues. To obtain a measure of the maximum endocardial thickness under which myocardial features could be visualized with this OCT system, measurements of endocardial thickness were obtained in regions where myofiber orientation and fibrosis could be seen in the en face OCT images. Out of 32 PVs, 23 veins had areas of sufficiently thin endocardial thickness to observe fiber orientation and fibrosis in the en face images of the venoatrial junction. In 3 LSPV, 2 LIPV, 3 RSPV, and 1 RIPV, fiber orientation and fibrosis were unable to be observed in the en face OCT images at the venoatrial junction. The endocardial thickness measurements are shown in Table 2. The average thickness under which fiber orientation and fibrosis could be observed was 0.28 ± 0.06 mm as measured from OCT, and 0.31 ± 0.08 mm as measured from histology, overall for all veins. The range of measurements were 0.15 to 0.43 mm and 0.12 to 0.47 mm for OCT and histology, respectively.

| Table 2. Endocardial Thickness, where Myocardium within OCT Imaging Depth |
|---------------------------------------------------------------|
| Thickness (mm) | LSPV (n = 15) | LIPV (n = 18) | RSPV (n = 15) | RIPV (n = 15) |
| OCT | 0.28 ± 0.04 | 0.30 ± 0.05 | 0.28 ± 0.07 | 0.28 ± 0.06 |
| | (0.2 to 0.39) | (0.22 to 0.41) | (0.15 to 0.39) | (0.20 to 0.43) |
| Histology | 0.30 ± 0.09 | 0.35 ± 0.05 | 0.30 ± 0.09 | 0.32 ± 0.07 |
| | (0.13 to 0.47) | (0.25 to 0.43) | (0.13 to 0.43) | (0.20 to 0.44) |

Values are given as mean ± standard deviation (range). LIPV: left inferior pulmonary vein; LSPV: left superior pulmonary vein; RIPV: right inferior pulmonary vein; RSPV: right superior pulmonary vein; OCT: optical coherence tomography.
4. Discussion

This study is the first, to the authors’ knowledge, to present comprehensive OCT imaging data of the human pulmonary venoatrial junction. This study provides imaging criteria for endocardium, myocardium, and venous tissues in the human LA and PVs. Myocardial sleeves, myofiber orientation, and fibrosis, as well as endocardial thickness and myointimal thickening, could be identified from the OCT images. OCT imaging of tissue architecture at the venoatrial junction could provide additional information of the AF structural substrate and potentially assist in guiding AF ablation procedures.

4.1 Tissue architecture of the venoatrial junction

The structure of the PV myocardial sleeves have been identified through histological studies to be highly complex. Ho et al. described an intricate arrangement of both circular and longitudinal myofiber bundles, with gaps and fibrosis commonly observed within the myocardial sleeves [2]. The structure of the PVs are also variable among different hearts and between individual veins. Saito et al. in particular identified some PVs having a more regular and aligned arrangement of myofibers, while others had non-uniform, crossing patterns [4]. Due to its complicated and unique structure, high resolution imaging is needed to fully capture the PV structural substrate. This study demonstrated that OCT is able to image similar myocardial sleeve features as seen from histology. In particular, OCT was able to visualize the orientation and density of myofibers and collagen fibers, which varied between veins. For instance, the parallel organization of myofibers and collagen fibers seen in Fig. 5 can be contrasted to the more complex organization seen in Fig. 7. Quantitative analysis showed that the myocardial and collagen fiber region seen in Fig. 5 had higher average local range, standard deviation, and entropy, but lower circular standard deviation of fiber angles than the region from Fig. 7. This was likely because the myocardial and collagen fiber region seen in Fig. 5 consisted of thinner, yet more organized fibers, in contrast to the larger and more disorganized collagen fibers seen in Fig. 7. Such analysis shows promise of quantitative metrics of fiber and fibrosis complexity from OCT images in the future. Finally, fiber orientation running in crossing directions were observed over different depths, as demonstrated in Figs. 3(e), 3(f), and 3(g).

4.2 Applications to the study of AF

There has been recent interest in the use of OCT to guide AF ablation therapy, specifically in imaging of ablation lesion formation [11,12,18,19]. In addition to lesion monitoring, however, OCT may be able to provide valuable information on the AF structural substrate. This study provided comprehensive OCT imaging data of the PVs, which can be used to assist the interpretation of human LA and PV OCT imaging in future studies, as well as inform the utility of OCT in defining the structural substrate underlying AF. Using OCT-integrated catheters, regions of complex fiber orientation or fibrosis imaged by OCT could be associated to electrical conduction patterns, in vivo. Given the varying biophysical properties of different tissue compositions, endocardial thickness, fibrosis, and myointimal thickening could also be taken into consideration when choosing ablation targets. OCT imaging of the PVs could also be applied to basic science applications, such as correlating the tissue structure identified by OCT to certain disease states, given a larger sample size of hearts with AF. The tissue structure identified by OCT data could also be incorporated into tissue-specific, electrophysiological models to better understand the influence of tissue microstructure on AF dynamics [20].

4.3 Influence of imaging depth

The main limitation of OCT is its imaging depth, which is determined by attenuation from optical scattering and absorption within the tissue [21]. Limitations are also posed by the
OCT system sensitivity, which was 100 dB at 28 kHz with a roll-off of 5 dB at 2.26 mm for this imaging system. In the case of cardiac tissue, imaging of myocardial features of interest such as fiber orientation and fibrosis is limited by the thickness and density of overlying endocardium and venous media. In this study, it was found that myocardial features could be visualized under overlying endocardial thicknesses averaging 0.28 mm for OCT measurements and 0.31 mm for histology measurements, respectively. The maximum measurements were 0.43 mm for OCT and 0.47 mm for histology. The thickness of the endocardium and venous media at the venoatrial junction has been determined from ultrasound and histology to range from 0.05 to 1.7 mm at the venoatrial junction and decrease to a range of 0.0 – 0.3 mm at 2 cm from the orifice towards the lung hilum [9]. Therefore, OCT is still applicable for a considerable range.

OCT enables identification of endocardial thickness with resolution that cannot be achieved by other commonly used cardiac imaging modalities. In areas of thicker endocardium, however, the lower boundary of the endocardial layer in OCT disappears, limiting the ability to accurately measure endocardial thickness. Nonetheless, the knowledge of when the endocardium exceeds a certain thickness could still be useful for understanding the properties of tissue being ablated. For \textit{ex vivo} applications, OCT imaging of the transmural atrial wall could be made possible through the use of optical clearing, imaging from both endocardial and epicardial surfaces, or thick sectioning of the tissue.

4.4 Limitations

Future studies are needed to more precisely assess the accuracy of OCT in identifying the lengths of the myocardial sleeves. In addition, this study was carried out \textit{ex vivo}. \textit{In vivo} imaging would pose additional challenges due to heart and catheter motion, as well as the need for real-time mapping and stitching to visualize large fields of views. Thus, future \textit{in vivo} studies would be required to determine if the same features described in this study can be visualized during ablation. Prior studies in swine, however, have demonstrated the capability of OCT-integrated catheters to image the atrial endocardium and myocardium \textit{in vivo} [11,12]. Only one out of ten donors in this study had a history of AF, and additional OCT imaging in hearts from donors with AF would be necessary to determine further applicability to AF ablation. During image pre-processing, it is possible that the contact glass surface of the sample z-Spacer, when completely adjacent with the endocardial surface, could not be distinguished from the endocardium, potentially affecting endocardial measurements in some cases. As an estimate of the potential error, the thickness of the z-Spacer surface seen in OCT was measured from one b-scan and found to be about 0.04 mm. Lastly, in this study, endocardial thickness measurements were sampled from three different points within the regions of interest to characterize the overall trend. Representative quantitative myofiber texture and angle measurements were also taken from selected ROIs. In the future, automated methods [10,16,22] can be more comprehensively applied to quantitatively evaluate endocardial thickness and other tissue features, such as myointimal thickening and fiber orientation, over the entire imaged region.

5. Conclusions

This study demonstrated that OCT can provide detailed visualization of tissue architecture at the human pulmonary venoatrial junction. Structural features such as myocardial sleeves, myofiber orientation, fibrosis, endocardial thickness, and myointimal thickening were identified from the OCT images. Imaging of detailed tissue architecture could assist in the guidance of ablation procedures and improve the understanding of the AF structural substrate.

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Disclosures

The authors declare that there are no conflicts of interest related to this article.

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