Spatial Distribution of Fibrosis Governs Fibrillation Wave Dynamics in the Posterior Left Atrium During Heart Failure

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Abstract—Heart failure (HF) commonly results in atrial fibrillation (AF) and fibrosis, but how the distribution of fibrosis impacts AF dynamics has not been studied. HF was induced in sheep by ventricular tachypacing (220 bpm, 6 to 7 weeks). Optical mapping (Di-4-ANEPPS, 300 frames/sec) of the posterior left atrial (PLA) endocardium was performed during sustained AF (burst pacing) in Langendorff-perfused HF (n=7, 4 μmol/L acetylcholine; n=3, no acetylcholine) and control (n=6) hearts. PLA breakthroughs were the most frequent activation pattern in both groups (72.0±4.6 and 90.2±2.7%, HF and control, respectively). However, unlike control, HF breakthroughs preferentially occurred at the PLAs periphery near the pulmonary vein ostia, and their beat-to-beat variability was greater than control (1.93±0.14 versus 1.47±0.07 changes/beats/sec, respectively, P<0.05). On histological analysis (picrosirius red), the area of diffuse fibrosis was larger in HF (23.4±0.4%) than control (14.1±0.6%; P<0.001, n=4). Also the number and size of fibrous patches were significantly larger and their location was more peripheral in HF than control. Computer simulations using 2-dimensional human atrial models with structural and ionic remodeling as in HF demonstrated that changes in AF activation frequency and dynamics were controlled by the interaction of electrical waves with clusters of fibrotic patches of various sizes and individual pulmonary vein ostia. During AF in failing hearts, heterogeneous spatial distribution of fibrosis at the PLA governs AF dynamics and fractionation. (Circ Res. 2007;101:839-847.)

Key Words: heart failure ■ atrial fibrillation ■ fibrosis ■ mapping ■ numerical simulations

Atrial fibrillation (AF) is the most common sustained arrhythmia in adults and is often associated with congestive heart failure (HF), which induces extracellular matrix remodeling involving atrial fibrosis and dilatation. Yet, how fibrosis contributes to AF mechanisms has not been thoroughly investigated. Previous experimental and clinical work has emphasized a role for fibrosis in arrhythmia maintenance. For example, interstitial fibrosis has been implicated in abnormalities during atrial pacing in dogs, and patchy fibrosis has been shown to cause activation delays in the human ventricle. In addition, chronic AF patients have an increased propensity to develop fibrosis at the posterior left atrium (PLA). However, the relation between AF dynamics at the PLA and the percentage and distribution of fibrosis in HF remains unexplored. Here we have characterized how AF frequency and dynamics on the endocardial surface of the PLA and pulmonary vein ostia (PVO) are affected by the amount, type (ie, diffuse versus patchy), and spatial distribution of fibrosis in failing hearts. It is our hypothesis that patchy rather than diffuse fibrosis contributes to wavebreak and intramural rotor formation, and governs the overall AF frequency and dynamics.

Materials and Methods

Heart Failure Model
All procedures were approved by the SUNY Upstate Medical University Committee for Humane Use of Animals. HF was induced in 7 sheep (15 to 25 kg) as previously described (see supplemental materials, available online at http://circres.ahajournals.org).

Isolated Heart Preparation
Both HF (n=10) and control sheep (n=6) were anesthetized (Na pentobarbital, 35 mg/Kg iv). The chest was opened through a midsternal incision. The heart was excised, placed in cardioplegic solution, and connected to a Langendorff apparatus for continuous perfusion with Tyrode’s solution at 200 mL/min (36 to 38°C; pH: 7.4; 95% O2, 5% CO2). The PLA endocardial surface was exposed through a minimal surgical opening in the left atrial appendage (LAA) avoiding any visible coronary branches. In Figure 1A, the incision lines are marked in a representative example (left panel) and

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the mapped area of the PLA that includes the 4 PVO is shown (right panel). In a recent study,7 we demonstrated that this procedure enables optical mapping of the PLA endocardium without any apparent damage to the circulation, and with AF frequencies and dynamics comparable to those observed in intact isolated hearts. In 7 HF and 6 control hearts, AF was initiated by burst pacing in the presence of 4 μmol/L ACh. In 3 additional HF hearts, nonsustained AF and atrial tachycardia (AT) were induced in the absence of ACh (see supplemental materials).

Optical Mapping Technique
This technique has been detailed elsewhere8 (see also supplemental materials). Briefly, no motion uncoupler was used (the separator exposing the endocardial surface mechanically restrained the PLA). After a bolus injection of 5 to 10 mL Di-4-ANEPPS (10 mg/mL), one CCD camera (DALSA, CA-D1-0128T-STDL) recorded fluorescence changes from an area of \( \approx 5 \text{ cm}^2 \) at 300 frames/sec to obtain 5-second movies (128×128 pixels).

Spectral Analysis, Dominant Frequency
Dominant frequency (DF) maps for each optical movie were calculated by applying a Fast Fourier Transform (FFT) to the fluorescence signal recorded at each pixel.9 To assess the location of the maximal DF (DFmax) domain at the PLA we superimposed the DF map and the corresponding picture of the field of view according to anatomical landmarks (PVO).

Histological Examination
Histological analysis of fibrosis was obtained from transmural PLA slices of HF and normal hearts (see supplemental materials for details).

Activation Patterns
Activation patterns analyses are described in detail in the online supplement.

Computer Simulations
2D computer models of characteristic PLA sections were developed based on realistic tissue and fibrosis geometry obtained from the histological study of control and failing hearts. Control and HF membrane ion kinetics of the myocytes and fibroblasts in the models were based on human atrial properties (for details, see supplemental materials).

Statistical Analysis
See online supplement for details on statistical methods. All results were expressed as mean±SEM; \( P<0.05 \) was considered statistically significant.

Results
Clinical, Electrocardiographic, and Echocardiographic Findings
All HF sheep manifested variable degrees of anorexia, lethargy, tachypnea, and gain of body weight, as well as episodes of atrial tachyarrhythmia at 182±6.0 bpm. As shown in supplemental Table I, significant clinical and echocardiographic hallmarks of systolic congestive HF were present in all HF sheep.

DF Distribution on the PLA
Figure 1 describes the distribution of DFs in the PLA and other regions of the atria. As shown in panel B, during sustained AF, the frequency of activation at the PLA was significantly larger than at the Bachmann bundle (BB) and the right atrial appendage (RAA) in both HF and control (\( P<0.05 \)). However, as previously,10–12 regional DFmax values at all locations (PLA, BB, and RAA) were significantly lower in the HF group compared with the control group (11.5±1.3 Hz at PLA; 8.0±1.0 Hz at RAA; *: \( P<0.05 \)). Representative examples of DF maps (left panels of Figure 1C and 1D for control and HF, respectively) with single pixel recordings (left traces) and bipolar electrograms (right traces) clearly support such a hierarchical organization. As shown by the maps, in control the DFmax domain (red) spanned the center of the PLA, whereas in HF DFmax was shifted toward the left inferior PVO. In control hearts, the DFmax area was 1.3±0.4 cm\(^2\), or 25.4±8.4% of the mapped PLA area. Superposition with an anatomical picture of the PLA similar to that in panel A revealed that in control the DFmax domain extended over the left PVO in 2/6 experiments and over both
left PVO and center of the PLA in 4/6 experiments. In contrast, in HF, the DFmax domain spanned an area of 1.1 ± 0.3 cm² (17.6 ± 5.5% of the PLA, P < 0.75) and localized mainly to the margin of the PLA, enclosing one or several PVO (5/7 experiments), or extended over the entire PLA (2/7 experiments). Interestingly, in all HF experiments, the signals recorded in the DFmax area were more fractionated than those recorded in other areas (panel D, trace c compared with trace d). Conversely, the signals from the DFmax area in control hearts were the most regular (panel C, trace a compared with b), in agreement with our recent report.7

Patterns of Propagation
As depicted in Figure 2A, during AF the most common pattern of activation was of a breakthrough type; only some examples of rotors were observed (2 in control and 1 in HF). For all the observed rotors, the period of rotation was equal to 1/DFmax (58.3 ± 8.3 ms in control and 79.9 ms in HF with 4 μmol/L ACh and 217 ms without ACh). Precisely, we observed 2 rotors in 2 different control animals, 1 rotor in one failing heart (in the presence of ACh 4 μmol/L) and one long-lasting rotor in the absence of ACh in one failing heart (see supplemental Movie I). The duration of the rotors varied widely: 2 examples with 1 to 2 rotations, 1 example with 2 to 3 rotations, and 1 example of a long lasting reentrant activity for several minutes. As for the breakthroughs, PLA outward breakthroughs were more frequent than inward breakthroughs during AF in both HF and control (P < 0.05), suggesting a driving role of the PLA in both groups. Diagrams of the spatial distribution of the breakthroughs are shown in Figure 2B for control and 2C for HF. Because of variations in heart dimensions, the diagrams metric is normalized to the distances between PVO. AF breakthrough waves in HF hearts tended to cluster peripherally, in closer vicinity to the PVO, compared with breakthrough waves in control (see supplemental Movies II and III for an example in control and HF, respectively). This observation is substantiated by comparing the radii of the areas with 25%, 50%, and 75% breakthroughs in panels B and C. Indeed, the area encircling 50% of HF breakthroughs was found to be on average about 30% larger than control (P < 0.01).

Histological Changes in the PLA
We evaluated the extent of remodeling and the distribution of fibrosis in the PLA of 4 HF and 4 control specimens by histological analysis using picrosirius-red staining. As shown in panels B and D of Figure 3, myocytes were larger in the HF group, as expected, and were often surrounded by increased extracellular matrix (ECM) compared with the control group (panels A and C). As previously observed in the ventricles,5 2 distinct patterns of fibrosis were distinguished in both the center (panels A and B) and periphery (near the PVO; panels C and D) of the PLA: diffuse (ie, small and unconnected

Figure 2. Activation patterns and spatial distribution of endocardial breakthrough sites at the PLA. A, AF waves patterns. *P < 0.05 Outward breakthroughs vs rotors and inward breakthroughs. B–C, Diagrams of PLA with superposed dots representing breakthrough sites in control (B) and HF (C). Each breakthrough color corresponds to a given experiment. Gray disks represent PVO (abbreviations as in Figure 1).

Figure 3. A–D, Micrographs showing distribution of fibrosis in the center (A–B) and periphery (C–D) of the PLA (picrosirius red staining). A and C, control. B and D, HF. E, Morphometric quantification of fibrous tissue content in control and HF specimens. *P < 0.001.
deposits of fibrous tissue) or patchy (ie, larger clusters of interconnected compact fibrous tissue). Further, quantification of the total amount of fibrosis by measuring the spatial extent of picrosirius-red stains revealed an overall significant ECM increment in the HF group compared with control at all 5 locations examined (23.4±0.4 versus 14.1±0.6%, P<0.001; Figure 3E). In addition, as shown in representative topographic maps of picrosirius-red staining of HF hearts (Figure 4A), the areas of patchy fibrosis were larger in the periphery (right panel) than the center of the PLA (left panel). As shown in Figure 4B and 4C, quantitative analysis of the area of fibrotic obstacles in all PLA specimens revealed a gradient in the amount of fibrosis in the HF specimens with greater ECM deposition in the periphery of the PLA. In 4 HF animals the average area of fibrosis in the periphery was significantly larger than in the center (0.163±0.052 mm² versus 0.036±0.003 mm², P<0.01) attributed mainly to the presence of a few patches with areas larger than 1 mm² (Figure 4B and 4C). The corresponding areas in control animals were 0.052±0.01 and 0.038±0.003 mm² for PLA periphery and center, respectively (P<0.01), with a remarkable absence of patches larger than 1 mm².

**Propagation of AF Waves**

Figure 5A presents 4 consecutive activation maps superimposed on an anatomical picture of the respective PLA in control (upper panels) and HF hearts (lower panels). In control, the direction of activation (black arrows), as well as the breakthrough sites, were highly recurrent from one AF wave to the next (maps 1 to 4). In contrast, the waves changed origin and direction on a beat-to-beat basis in the failing heart (maps 1’ to 4’). Classification of PLA waves into 7 subgroups based on their pattern of propagation (see Methods) allowed calculation of the rate-normalized changes in the AF wave patterns. Figure 5B summarizes such beat-to-beat changes and demonstrates a significantly larger degree of variability in HF than control (1.93±0.14 versus 1.47±0.07 changes/ [beats/sec], P<0.05). Further, despite the lower activation frequency in HF hearts, we observed a tendency toward an increased number of singularity points in the DFmax domains.

![Figure 4. A, Topographic maps of fibrosis in the center (left) and periphery of the PLA (right) in a representative example (green dotted lines delineate areas of epicardial and endocardial red pixels that were excluded from analysis; see supplemental materials for further details). B, Average areas of fibrotic obstacles in the center and periphery of the PLA of control and HF hearts. C, Histogram of fibrotic obstacle areas (same color code as in B).](image-url)
or at their borders (data not shown) in HF (4.4±2.2/experiment, range 0 to 13) compared with control (0.5±0.3/experiment, range 0 to 2, P=0.13).

Numerical Predictions

**PLA Tissue and Fibrosis**

As illustrated in Figure 6, we developed 2-dimensional computer models of transmural PLA sections incorporating human atrial ionic models for control and HF conditions,3 3 different realistic PLA boundary geometries (G-I, G-II, and G-III) and 3 different spatial distributions of fibrosis (patchy, diffuse heterogeneous, and diffuse homogeneous).3 Please refer to the supplemental materials for a detailed description of the methods used.

**Intramural Propagation Patterns**

Figure 7 illustrates how HF may affect propagation. For this set of simulations a comparison between control and HF dynamics was done by using the same boundary geometry of HF PLA (G-I, see Figure 6), while changing the fibrosis distribution. This strategy was preferred over using the actual anatomical geometry of the control heart (G-III), to focus only on the functional effect of the difference in the fibrotic tissue quantity and architecture between the 2 hearts. In control, S1-S2 stimulation induced intramural reentry (white arrows) at 23.4 Hz (Figure 7A left, supplemental Movie IV). This rotor drifted rapidly toward the right and terminated at the PLA boundary after 4 rotations, without any apparent conduction impairment, as shown by the endocardial (red dashed line) time-space plot (TSP) that was used to compare these data with the experimental results. In contrast, in HF, S1-S2 induced a slower intramural reentry (5.9 Hz) around a central patchy fibrotic area that perpetuated for the entire 2-second episode (Figure 7B left, supplemental Movie V). Moreover, the slower reentry in the HF model generated waves that fragmented when colliding with fibrotic obstacles (white dots). The TSP shows an endocardial breakthrough at location 1 and a delay in the septal-to-lateral propagation at location 2, produced by a large fibrous patch.

To address the possibility of an underlying focal source for the endocardial breakthroughs (see Figures 2 and 5), artificial pacing was applied to the center of the model (pulse symbol) at 6 and 20 Hz, for the HF and control conditions respectively, to represent experimental activation frequencies. In control (Figure 7A, right; supplemental Movie VI), the regularly paced waves propagated uninterrupted, producing a stable endocardial breakthrough site (location 3). On the other hand, as depicted in Figure 7B (right panels, supplemental Movie VII), HF resulted in propagation disturbances with singularity points clustered about fibrotic obstacles. For example, a wave attempting to cross a densely fibrotic region from left to right underwent unidirectional block after the first stimulus (black arrow at 519 ms), resulting in sustained reentry (5.9 Hz) around a large patch of fibrosis and repetitive endocardial breakthroughs at location 4. Then, at 1740 ms, unidirectional block closer to the septum (not shown) gave rise to a much faster rotor (21.5 Hz) that persisted for the remainder of the simulation. In this case, large propagation delays induced by fibrotic obstacles near the lateral wall led to endocardial breakthroughs at location 5. Also, in accordance with the experimentally observed variability in HF breakthrough patterns, the onset of the second intramural reentry shifted the location of the endocardial breakthrough from site 4 to 6. Overall, the simulations presented in Figure 7 predict that regardless of whether the AF mechanism is reentrant or focal, the large size of the fibrotic patches in HF is the major factor responsible for the decreased rate of AF waves.

**Peripheral PLA and PV Activity**

We further examined the relationship between fibrosis distribution and endocardial breakthrough pattern in a different

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**Figure 5.** A, Representative activation maps of 4 consecutive AF waves at the PLA in HF and control groups. The maps are superposed on a color picture of the preparation. The patterns of propagation are highly recurrent in control (top) in comparison to HF (bottom). Abbreviations as in Figure 1. B, Number of changes (normalized to the frequency) of wave pattern during AF waves in control and HF hearts. *P<0.05.
geometry model (PLA geometry G-II, Figure 6), constructed from a transmural slice that included a PVO of a failing heart (Figure 8A). We decided to focus specifically on the LIPV ostial area because of our experimental observation that PVOs, characterized by large patches of fibrosis, are the preferential region for breakthroughs (see Figures 2 and 4). Figure 8B presents snapshots of the electrical activity at selected time frames. S1-S2 led to intramural figure-of-eight reentry (22.7 Hz) in an area where fibrosis was sparse (frames 94 and 122 ms). Eventually, after 2 full rotations, the left arm of the figure-of-eight shifted toward the LIPV. This resulted in counterclockwise reentry around the PVO at a slower frequency of 5.8 Hz (frames 288, 359, and 423 ms and supplemental Movie VIII). Concomitantly, the right arm of the initial figure-of-eight generated a wave that traveled downward and to the left between a very elongated fibrous tissue septum and the epicardium. This wave vanished when it collided with the lowermost border of the sheet, which rendered the PV reentry dominant. It is noteworthy that the two different reentry frequencies that were measured in this simulation were similar to those obtained for the first geometrical model, in which the HF condition and pacing stimulation were applied. Also, similar to the previous simulation, the rapid change of reentry pattern was accompanied by a rapid change of endocardial breakthrough location, as can be seen in the endocardial TSP of figure 8B (transition from location 1 to 2). Two representative examples of local electrograms (a and b) are shown in Figure 8C, demonstrating fractionation and irregularity of the membrane potential of myocytes near fibrotic tissue. For comparison, we used a model with the same HF ionic properties and tissue geometry, but only diffuse fibrosis (supplemental Movie IX). In this case a reentrant wave formed after S1-S2 stimulation. It did not anchor but drifted toward the PLA center where it terminated after 5 rotations by collision with the epicardial boundary.

**Discussion**

**Major Findings**

We evaluated the consequences of HF remodeling on PLA AF dynamics. Specifically, we examined how the heterogeneous HF architecture of PLA fibrosis alters AF wave propagation. Our work represents a substantial extension of previous studies demonstrating that fibrosis provides a substrate for AF24 because of atrial conduction disturbances,3,15 and showing that AF dynamics in HF differs from that in normal hearts.10 A major result of these combined experimental-simulation results was that whether the mechanism of atrial fibrillation is reentrant or focal, the large size of the fibrous patches in heart failure provides the major factor responsible for different dynamics of AF waves in HF versus control hearts. Here, we demonstrate for the first time that (1) during
AF, endocardial breakthroughs waves, that are by far the most common activation pattern at the PLA in both normal and HF groups, are more variable in HF hearts and distribute more peripherally, closer to the PVO. (2) The amount, distribution, and type of fibrosis are significantly different in HF and control hearts. Importantly, in HF the architecture of fibrosis in the PLA consists primarily of patches with the larger fibrotic obstacles found preferentially in the vicinity of the PVO. (3) Both large PLA fibrous patches and the PVO act to anchor reentrant circuits and impair wave propagation to generate delays, wavebreaks and signal fractionation.

**Frequency of Activation and Location of AF Sources**

Our experiments demonstrate that PLA sources maintain AF in both normal and HF hearts. In both groups, AF waves were visualized as breakthroughs traveling from inside to outside the field of view (Figure 2) with a significantly higher DF in the PLA compared with other atrial areas (Figure 1). These data corroborate previous animal and human findings confirming that the PLA plays a key role in maintaining AF activity. Specifically, Wu et al have shown in patients in permanent AF and organic heart disease that epicardial AF dynamics was characterized by rapid repetitive activations originating near the PVOs. Also, our numerical simulations show that the activity in HF hearts revolved around obstacles, formed by either non-excitible patches of fibrosis or one of the PVO, with a cycle length that depended on the obstacle size (Figures 7B and 8), and was found significantly larger in HF.

**Mechanism of AF Maintenance**

Both reentry and focal activities at or near the PV area have been shown to sustain AF in the atria of failing dog hearts, and in the presence of ACh. Our experiments demonstrate that the periostial area (PLA periphery) harbors the largest fibrotic obstacles (Figures 3 and 4), the most breakthrough sites (Figure 2), and the highest frequency domains. Furthermore, our computer simulations illustrate that the largest fibrotic patches and the PVO are potential anchoring sites for “micro-anatomical” reentry. Thus, our results suggest that the fibrillatory activity in our experiments was maintained by intramural reentry centered on fibrotic patches and that it appeared at the PLA as breakthroughs. Additional measurement of PLA myocardial thickness, transillumination experiments, as well as a 3D simulation of an intra-atrial scroll presented in the supplemental materials further support this assertion. Nonetheless, we cannot fully exclude a spontaneous pacemaker or triggered activity mechanism possibly interacting with an evolving reentry, as a source of AF. This was supported by the simulated triggered activity in our numerical simulations that yielded reentry impulse propagation disturbance and anchoring shift (see Figure 7). Altogether, in HF, the remodeled substrate of the PLA seems to be more important for maintaining AF than the nature of the source, whether reentry or triggered activity.

**AF Dynamics in HF and Control: Role of Fibrosis Amount and Architecture**

Both human and animal studies have described the changes of AF dynamics induced by HF. For instance, local conduction abnormalities attributable to interstitial fibrosis have been reported in a HF model in dogs, and patients in chronic AF have demonstrated increased interstitial fibrosis in the PLA in comparison with the LAA and also with the PLA of sinus rhythm patients. However, to the best of our knowledge, no previous study has addressed the role of the architecture of interstitial fibrosis on PLA impulse propagation during AF.

Figure 7. Computer simulation in PLA transmural slices for control (A) and HF (B) conditions. A, Snapshots at several timeframes for cross-field stimulation (left) and pacing at a frequency of 20 Hz (right), as well as endocardial time-space plots (constructed for the segment marked by the red dashed line as in Davidenko et al). Colors indicate transmembrane voltage from low (blue) to high (red) B, Snapshots at several timeframes for cross-field stimulation (left) and pacing at a frequency of 6 Hz (right), as well as endocardial time space plots. The site of unidirectional block (ub.) is pointed by a black arrow. White circles on the upper voltage maps of panel B indicate sites of wavebreak.
We demonstrate here for the first time that in the PLA major changes in AF dynamics are caused by an increased amount and a different architecture of fibrosis caused by HF. Interestingly, the fibrotic patches govern AF dynamics at the PLA by playing various roles that are sometimes conflicting in nature. First, because of their ability to act as microanatomical obstacles they have the propensity to anchor reentrant sources. However, the patches need not be confluent to be the central pivoting attachment of reentry. In additional simulations presented in the supplemental materials, we demonstrate that a simple heterogeneous distribution of diffuse fibrosis can attach a reentry (see supplemental Figure VIA and VIB and supplemental Movies X to XIII) at a specific PLA location. Second, the existence of large fibrotic patches increases the likelihood of low frequency reentrant activities. As shown in Figures 1B and 4B, we observed experimentally larger fibrotic obstacles in failing hearts and correspondingly a lower DFmax. Such observations are supported by the numerical experiments shown in Figure 7A and 7B. Third, the patches of fibrosis increase dramatically the variability and complexity of the patterns. They are, for instance, responsible for propagation delays and unidirectional blocks (see Figure 5A and 5B and Figure 7B). However, it should be noted that the fact that the DFmax is lower in HF than in control does not exclude some short transitional episodes of fast reentrant activity. In fact, their ability to anchor to obstacles of different size and shape make transient reentrant sources of various frequencies likely to appear sequentially (see Figures 7B, right panel and 8B and supplemental Movies VII and VIII) or even to coexist (see supplemental Figure VIA and supplemental Movie X). Fourth, in good accordance with the experimental results (Figure 2B and 2C), the simulations predict that the presence of PV ectopic activity related to stretch-related lengthening of the myocytes might reproducibly initiate a reentrant circuit that would anchor to fibrotic patches adjacent to the PVO.

Clinical Implications
Whereas AF ablation for HF patients has been proven feasible, the difference of AF wave dynamics at the PLA in those patients had not been previously explored. In this work, we present experimental and computational evidence suggesting that the sites of AF sources and their related endocardial breakthrough sites appear toward the periostial area of the PLA, close to large fibrous patches. These data open a new perspective for improvement of radiofrequency catheter ablation procedure for HF patients, suggesting that in HF the outer PLA and areas with large fibrotic obstacles are critical for AF maintenance.

Limitations
Sustained AF was induced in the presence of 4 μmol/L ACh. In the absence of ACh in failing hearts we usually obtained AT and nonsustained AF episodes. However, detailed analysis of the data obtained in 3 hearts demonstrated that AF-AT
changes in HF hearts, such as anisotropy, heterogeneous muscle fibers and fibrosis, which greatly slows the conduction of atrial impulses. This can lead to a single spiral wave that organized fibrillation and can be stabilized by a rotor anchored in the posterior left atrium. In the same way, fibrillation can be maintained by a rotor anchored in the posterior left atrium.

Although we show that fibrosis plays a strong role in determining AF dynamics, we cannot exclude additional mechanisms of frequency slowing and/or of AF dynamics changes in HF hearts, such as anisotropy, heterogeneous muscle fibers and fibrosis, which greatly slows the conduction of atrial impulses. This can lead to a single spiral wave that organized fibrillation and can be stabilized by a rotor anchored in the posterior left atrium. In the same way, fibrillation can be maintained by a rotor anchored in the posterior left atrium.

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**Disclosures**

None.

**References**


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METHODS

Heart Failure Model
A unipolar pacing lead (St. Jude Medical, Inc.) was inserted into the right ventricular apex; a pacemaker was implanted and programmed to capture the ventricles at 220 beats/min for 6-7 weeks. For follow-up, clinical signs of heart failure (HF: lethargy, edema, dyspnea, gain of body weight, loss of appetite) and transthoracic echocardiographic evaluations (Phillips, Sonos 5500) were monitored weekly. In particular, echocardiographic left atrial and left ventricular dimensions in the parasternal long-axis view and left ventricular ejection fraction (Teicholz formula, M-mode) were recorded. Once parameters became compatible with HF, pacemakers were stopped and hearts were collected 48 hours afterwards.

Optical Mapping Technique
This technique has been described in detail elsewhere. Briefly, a bolus injection of 5 to 10 mL of the dye Di-4-ANEPPS (10 mg/mL) was used to image the fluorescence resulting from changes in membrane potential. Three tungsten-halogen light sources, two for epi-illumination and one for trans-illumination, were utilized to homogeneously excite the fluorescence of the potentiometric dye. Sustained AF (>5 minutes) was induced by burst pacing in the continuous presence of acetylcholine (ACh, 4 μM). The emitted signals from a posterior left atrial (PLA) area of ~5 cm² were captured by one CCD camera (DALSA, CA-D1-0128T-STDL) at 300 frames/sec to obtain 5-second movies (128×128 pixels). Three movies were recorded in each experiment and bipolar
electrograms were obtained continuously from two locations, one in the septal portion of Bachman’s bundle (BB) and the other in right atrial appendage (RAA).

**Activation Patterns**

Activation times for each pixel were determined using thresholding techniques with a non-linear best fit of the upstroke and then color-coded to generate activation maps. ³,⁴ Phase movies were obtained as previously⁵ and the number of phase singularities in one second of AF in each movie was manually determined. AF waves were classified into 3 patterns: (i) outward breakthrough, a wave appearing at the PLA and propagating toward the periphery of the field of view, (ii) rotor, a spiral wave lasting more than one rotation, and (iii) inward breakthrough, a wave traveling from the margin toward the center of the mapped area (the margin was defined as the outer ~15% of the field of view). In addition, in one movie per experiment, the breakthrough sites (defined as the area of the wave center in its first frame of occurrence) were plotted on schematic PLA diagrams according to their relative distance from PVO. The specific direction of propagation was then manually analyzed wave-by-wave to further classify each breakthrough into one of 7 subgroups, depending on the main direction of wave propagation: symmetric (i.e., equal propagation in all directions), lateral to septum, septum to lateral, inferior to superior, superior to inferior, colliding waves and rotors. We also calculated the rate of change of directional patterns (normalized to frequency) in all recorded movies.

**Spectral Analysis, Dominant Frequency**

As previously described, to construct dominant frequency (DF) maps of each optical movie a fast Fourier transform (FFT) was applied to the fluorescent signal recorded at
each pixel. The frequency depicting the maximum power was considered to be the DF and assigned a color between dark blue and red according to its value. The area with the maximal DF (DF\text{max} domain) was defined as the area corresponding to the highest DF in that map. To assess the exact location of the DF\text{max} domain at the PLA we superimposed the DF map and a corresponding digital picture of the field of view according to anatomical landmarks (pulmonary vein ostia; PVO).

\textit{Histological Examination}

After optical mapping, hearts were immersed in 10% neutral buffered formalin, processed for paraffin embedding and 4 µm sections were obtained from blocks spanning the PLA. Quantitative evaluations of fibrosis were performed on images obtained from 5 different locations in the PLA (left superior pulmonary vein area, left inferior pulmonary vein area, right superior pulmonary vein area, right inferior pulmonary vein area and center of the PLA) after staining with picrosirius red. Images were photographed using either 10x or 2.5x objective, with either a digital 3-chip color CCD camera (DC-330, DAGE-MTI) installed on a Zeiss Axioplan2e microscope or a 12bit color CCD camera (Qicam, Qimaging) installed on a Zeiss ACIO imager A1 microscope. Areas occupied by fibrotic tissue were automatically measured with Bioquant’s Life Science package software by employing a color thresholding technique. Perivascular, endocardial and epicardial fibrosis were excluded from the analysis by setting regions of interest. To determine the sizes of individual fibrotic patches, sections from both the center and peripheral PLA with comparable planes of section were identified and overlapping images spanning the entire section were acquired using the Bioquant Automated Imaging toolkit and Topographer plugin (R & M Biometrics, Inc. Nashville, TN). Fibrotic tissue
was identified using color thresholding and the area occupied by individual fibrotic patches was automatically measured as described above. For patches that extended across multiple fields of view, the separate areas were summed manually and the autoexclude feature of the software was employed to ensure that selected pixels were measured only once.

**Computer Simulations**

Computer models of the PLA with realistic 2D geometry, fibrosis distribution and ionic kinetics were employed (see figure 6 of the main manuscript).

**Boundary Geometries**

We utilized 2 realistic HF PLA geometries. A general transmural PLA geometry (geometry G-I, see figure 6, upper left panel) and also the left inferior PVO area of a HF heart (geometry G-II, see figure 6, middle left panel), reflecting our previous observation that the latter region was characterized by large patches of fibrosis and the preferential location of breakthroughs. As a control, a geometry of a normal heart (geometry G-III, see figure 6 lower left panel) was implemented. The 3 geometry models were extracted out of histological sections of control and HF PLAs, and were sampled into a spatial resolution of $\Delta x=\Delta y=45.5\mu m$ (for G-I and G-III) and $\Delta x=\Delta y=95\mu m$ (for G-II).

**Fibrosis Distribution**

The two distinct fibrosis distributions - patchy and diffuse - were modeled as follows (see also figure 6, middle panels):
1. Patchy distribution

Patchy distribution was realistically extracted of picrosirius stained histological sections, where the dyed extra-cellular matrix (ECM) tissue could be easily distinguished by its characteristic reddish color content. An automatic segmentation procedure was applied, where each of the image pixels was classified as either external to tissue, myocyte or fibrotic tissue using a color threshold. The segmented image was passed through an erosion filter with a square structure element of size 3 pixels, in order to emphasize the characteristic patchy fibrosis of a HF heart.  

2. Diffuse fibrosis

Diffusive fibrosis was defined as very small deposits of ECM, in the order of 1 computational cell (~50um), and was artificially added to our models, usually with random distribution with a pre-defined total number of fibrosis cells. In several simulations, however, we additionally investigated the effect of homogeneity in the diffuse fibrosis distribution, and thus compared random versus uniform distribution of fibrosis, in which the distance between every fibrotic cell and the 4 closest fibrotic cells was fixed.

HF and Control Conditions

To realistically mimic the HF and control conditions, and in accordance with our histological findings, we refer to the HF condition as a combination of half patchy and half diffuse fibrosis and to the control condition as only diffuse fibrosis. The final fibroblast/myocytes area ratio was approximately 10% in HF condition and 5% in control.
Ionic Models

The temporal values of the transmembrane potential were calculated numerically by solving the discrete standard reaction-diffusion equation, assuming isotropic tissue and Neuman boundary conditions, and incorporating human atria ionic kinetics, including an $I_{kACh}$ formulation from Kneller et al. For the HF model, the ionic channel properties were modified according to Li et al. The changes included decreases of $I_{Ca}$ and $I_{Ks}$ by 30%, a decrease of $I_{lo}$ by 50%, and an increase of the sodium/calcium exchanger current by 45%. In an additional simulation we investigated AF PLA dynamics in HF condition including a stretch-activated channels (SACs) model. We implemented a commonly accepted IV relationship of SACs as described by Kamkin et al. in human atrial cells. Referring to this work, the level of 4 μm, corresponding to moderate stretch, was selected and related to the increased LA diameter measured in HF hearts (see online Table 1). For all these models, the fibrotic grid cells were computed as non-excitable media, using the following passive differential equation for describing the local potential, $V$:

$$\frac{\partial V}{\partial t} = -\frac{V - V_r}{CR_f} + \nabla \cdot (D_f \nabla V)$$

where $C$ [F] is the capacitance of a fibroblast, taken to be equal to that of a myocyte (25 pF for the present grid size), $V_r$=-15.9mV is the resting membrane potential of a fibroblast, $R_f$=4.1GΩ is the passive resistance of a fibroblast, and $D_f$ [mm² ms⁻¹] is an average diffusive coefficient of the fibrotic tissue. $D_f$ was taken as a fraction of $D$; i.e. $D_f = \kappa_f D$ represents electrical coupling within fibroblasts. At the boundary between fibrotic and myocardial tissue, a geometrical average of diffusion coefficients was taken,
to maintain current flow conservation, i.e.,

\[
D_{\text{fibroblast/myocyte}} = \frac{2D_{\text{fibroblast}}}{2D_{\text{fibroblast}} + D_{\text{myocyte}}} = \frac{2\kappa_f}{1 + \kappa_f} D
\]

We set \( \kappa_f = 0.1 \) to representing some low electrical coupling between fibroblasts and between the fibrotic tissue and its surrounding myocardial tissue. For comparison, we repeated all simulations with interchanged kinetics, i.e. employing the control ionic kinetics for the HF fibrosis distribution (10% patchy and diffuse fibrosis) and the HF ionic kinetics for the control distribution of fibrosis (5% diffuse fibrosis).

**Numerical Protocol**

Two types of stimulations were applied: 1) S1-S2 cross-field stimulation, and 2) pacing stimulation with frequencies of 6 and 20 Hz for the HF and control, respectively, which represent realistic activation frequencies observed experimentally.

**Statistical Analysis**

Unpaired \( t \)-test was used to compare clinical and echocardiographic parameters and the average area of fibrotic obstacles between center and periphery of the PLA. For characterization of the spatial distribution of breakthrough sites the Boltzmann equation was best-fit to each heart and the radii of their 50-percentile were compared with a t-test after confirming normal distribution (Shapiro-Wilk test). We compared (i) DF values, (ii) percentages of fibrosis at 5 PLA locations (LSPV, LIPV, RSPV, RIPV, Center), (iii) percentages of diffuse and patchy fibrosis patterns in control and HF conditions (iv) average number of each wave type, (v) number of wave pattern changes. The statistical designs for each respective data comparison were: (i) 2 (condition; control or HF) \( \times 3 \) (location; PLA, BB or RAA) \( \times 3 \) (movie; M1 through M3). Frequency data were also
compared by use of a dependent-measures ANOVA, with 2-tailed $\alpha=0.05$. In this analysis, we used a priori contrasts comparing frequency data in the DFmax area with those in the BB and RAA regions, (ii) 2 (condition; control or HF) x 5 (location; L1 through L5), (iii) 2 (condition; control or HF) x2 (architecture; diffuse or patchy) (iv) 2 (condition; control or HF) x 3 (wave type; breakthrough, rotor or outwave), (v) 2 (condition; control or HF) x 3 (movie; M1 through M3), dependent-measures ANOVA. For all repeated ANOVA comparisons we set 2-tailed $\alpha$ to 0.05. We used Mauchley’s test of sphericity on our repeated observations and interpreted Huynh-Feldt-adjusted significance values in cases in which our data required corrections for non sphericity. A non parametric Kolmogorov-Smirnov test was employed for group comparisons of areas of fibrosis patches in the center and periphery of the PLA in normal and HF hearts. All results were expressed as mean±SEM and $p<0.05$ was considered statistically significant.

RESULTS

Experimental Results

Breakthrough Sites on the PLA.

Figure OS1 shows all the PLA regions that were analyzed histologically in 3 hearts (A, B and C respectively). For each heart, a star indicates the areas that harbored the largest number of breakthroughs as shown also in figure 2C of the main manuscript. These figures enabled us to compare ostial (panels A and B) and central PLA (panel C) areas. As expected, most of the breakthroughs occurred where the largest fibrotic obstacles were observed. These data support our contention that large fibrotic obstacles have the propensity to attract and anchor in their vicinity the electrical sources of AF.
**AF dynamics in the absence of ACh**

As presented in the main article, in 7 HF hearts AF was maintained in the presence of 4 μM ACh. To rule out the possibility that ACh, rather than fibrosis, determined the dynamics of AF in this HF model, we investigated AF dynamics in 3 additional failing hearts in the absence of ACh, as shown in Figure OS2. Panel A is a diagram of the PLA similar to that shown in figure 1 of the main paper. Breakthrough sites are represented by color dots, each color corresponding to a given experiment (n=3). In all experiments, the majority of breakthrough sites were located at the periphery of the PLA. Panels B and C show sequential phase maps obtained from a representative experiment during an AF episode. Panel D shows the corresponding RAA, LSPV and LAA electrograms. In B, repetitive breakthroughs near the RSPV eventually led to the formation of a stable, long-lasting rotor that anchored in the vicinity of the LSPV ostium (see movie 1). Similar results were obtained in all three experiments. Except for the long-lasting rotor shown in figure OS2B-D, for the most part, in the absence of ACh we usually induced atrial tachycardia and non-sustained AF episodes in the majority HF hearts. Yet, detailed analysis of the data in all three hearts demonstrated that while the frequency of AF-AT was slower, the overall dynamics in the absence were very similar to those in the presence of 4μM ACh. Altogether, these data demonstrate that the dynamics of atrial arrhythmias in this heart failure model are governed by fibrosis distribution rather than ACh concentration.

**Mechanisms of breakthrough wave occurrence**

PLA breakthroughs were the most frequent activation pattern seen in our HF model during AF. Two possible electrophysiological mechanisms may account for such
breakthroughs: 1. an intramural scroll wave whose filament orients parallel to the endocardial wall; 2. rapid, spontaneous pacemaker or triggered discharges from a localized focus. One relatively straightforward way to differentiate between these two arrhythmogenic mechanisms would be to carry out simultaneous optical mapping of the epicardium and endocardium. Unfortunately, the large density fatty tissue on the epicardial side of the central PLA interfered with the optical signal to the extent that it precluded us from using such an approach.

**Measurements of PLA thickness**

As a first step in our effort to elucidate the nature of the endocardial breakthroughs, we first measured the thickness of the PLA myocardial wall in 8 normal hearts. A scalpel was used to make cross-sectional cuts; wall thickness was measured at 5 separate locations using a ruler under a stereoscopic microscope (2.5x). We found the overall average thickness to be 3.2±0.2 mm; the average minimal thickness was 1.7±0.2 mm (range 1.5-2.5 mm), in the inferior part of the PLA; the average maximal thickness was 4.7±0.4 mm (range 4.0-6.0 mm), close to both ostia of the superior PVs. Hence, given the fact that during AF microreentrant sources in the PLA have an average core diameter of <2 mm, such thickness seems to be sufficient to allow enough “elbow room” to harbor a stable intramural scroll wave whose filament is parallel to the endocardial surface.

**Transillumination experiments**

To further investigate whether or not an intramural PLA scroll wave is responsible for the breakthrough activity, we implemented a transillumination technique at the transition
between the PLA and LAA based on a previously described method.\textsuperscript{16,20} This approach allows detection of areas of low signal amplitude which, when correlated with the patterns of propagation, can be related to the presence of intramural scroll filaments. In 4 such experiments we introduced a light guide into the intact left atrium through a small left ventricular opening and across the mitral valve ostium. We focused the filtered light on the PLA-LAA transitional area, while recording the transilluminated fluorescence from the epicardial surface. This transitional area was chosen in an effort to avoid the epicardial fatty region of the central PLA and allow high quality optical recordings. From the resulting signal, we obtained movies for the construction of amplitude maps and isochronal maps, as well as time-space plots of the activity. Panel A of figure OS3 shows data from a representative 1.5-second episode. The isochrone (top) and amplitude (bottom) maps disclose the presence of two counter-rotating reentrant wavefronts. The time-space plot, constructed along the horizontal broken line on the map, reveals several transient episodes of reentrant activity with the typical christmas-tree like pattern\textsuperscript{18} (highlighted by yellow dashed rectangles and by the magnified red inset). The presence of an area of low potentiometric dye fluorescence in the center of the time-space plot during each of these transient episodes is strongly suggestive of reentrant activity.\textsuperscript{18} In all experiments, such patterns were separated from each other by episodes of variable duration in which spatiotemporally periodic waves entered the field of view from one or more directions. Altogether, the data shown in panel A indicate the presence of sustained reentrant activity that transiently entered the field of view, somehow shifted its position to disappear, and again reappeared intermittently over a period of 1.5 seconds. The fact that in the epifluorescence recording experiments only breakthrough patterns were seen on the endocardium is compatible with intramural reentry.
The computer simulations of 3D reentry shown in panel B and C aid us in the interpretation of such data. A single stationary scroll wave is generated in a cube of cardiac muscle. On the left, the organizing center of the scroll wave (i.e., the filament) is perpendicular to the epicardial wall. Epicardial transillumination signals were computed according to Baxter et al. \(^{16}\) for simulated 0.5 sec. activation. A time space plot constructed along the black line reveals the christmas-tree like pattern of sustained reentry. \(^{18,20}\) On the lower panel, the scroll wave shifts its position and the filament now is no longer in the field of view. Under these conditions we no longer see any evidence of reentry on that wall. Only spatiotemporally periodic breakthroughs are seen with each rotation of the scroll in the time space plot. Note, however, that the experimental isochrone map of panel A shows two counter-rotating reentrant waves rather than only one. As illustrated by the bottom cartoon in panel C, we interpret this pattern as being compatible with the presence of a U-shaped filament. This clearly would result in figure-of-eight reentry on the epicardial surface with breakthroughs emerging only on the endocardial wall. A christmas tree-like pattern of reentry would be observed on a time space plot drawn for the plane crossing the u-shaped scroll perpendicular to the epicardial wall. Alternatively, as shown by the top cartoon in panel C, the figure-of-eight reentry could result from the co-existence of two counter-rotating transmural scroll waves. However, this interpretation would be incompatible with either the emergence of breakthroughs with absence of evidence of reentry on the endocardium, or the christmas-tree like patterns in the time space plots. Altogether, our experimental observations and accompanying simulations strongly suggest that, even if transiently manifest, relatively long-lived non-stationary intramural scroll waves occurring within the confines of the
PLA wall are the underlying mechanism of AF, as well as of the endocardial breakthrough patterns observed in this HF model.

**Numerical simulations**

*Effect of Ionic Remodeling in HF*

We determined whether the observed changes in activation rate and pattern between HF and control conditions (as presented in manuscript figures 7 and 8) could be solely attributed to the different ionic models. Thus, the simulations were repeated with the ionic kinetics interchanged, i.e. the HF fibrosis distribution model was run with the control kinetics, and vice versa. The results are given in online supplemental figures OS4 and OS5. Figure OS4 demonstrates that employing the HF kinetics with the control geometry (diffuse fibrosis) for the entire transmural PLA model results in activation patterns similar to those seen in figure 7A. Close inspection revealed only minor changes in transmembrane voltage traces (not shown). The results for the HF fibrosis distribution using the control kinetics (figure OS4-B) showed similar activation pattern to figure 7B when S1-S2 stimulation was applied. On the other hand, pacing gave rise to activation pattern changes that were different from those in figure 7B. Reentry was stable, with a frequency of ~5.3 Hz. Closer inspection revealed that the unidirectional block that was present close to the pacing location when the HF kinetics were applied (marked in figure 7B) ceased to exist when the control kinetics were used. The results of the simulations of the LIPV ostium area with the interchanged ionic models are given in figure OS5, which shows an AF wave propagation pattern similar to that seen in figure 8, with a slight increase in the activation rate (less than 1 Hz). Thus, the simulations show that the unique architecture of the peripheral transmural PLA, with the PVO and large fibrotic patches in
their vicinity favor intramural reentrant activity at relatively low rates and explain the experimental observations of increased propensity of constantly changing peripheral breakthrough sites and patterns in HF. As such, ionic remodeling seems to contribute little to the decreased reentry frequency and variable AF activation patterns seen in HF.

**Effect of Heterogeneous Distribution of Diffuse Fibrosis**

Our initial simulations (see figures 7, 8, OS4 and OS5) explored the role of patchy fibrosis distribution and demonstrated its ability to impinge on normal impulse propagation and generate delays, unidirectional blocks and anchored reentrant activity. We also explored the role of the homogeneity of diffuse fibrosis distribution by utilizing both uniformly (homogeneously) and randomly (heterogeneously) distributed diffuse fibrosis. We performed simulations with 10% of diffuse fibrosis with uniformly and randomly distributed fibrosis (see online methods above), utilizing geometry G-I with HF ionic kinetics. The behavior clearly depended on the homogeneity of fibrosis distribution. As illustrated by panel A of Figure OS6 (left), when the 10% fibrosis was randomly distributed, S1-S2 stimulation initiated a reentrant source at 25.5 Hz. Subsequently, at 340 ms, a wavebreak gave rise to a second reentrant source whose frequency was 28.3 Hz (see also online movie 10). The 2 reentries coexisted throughout the remainder of the simulation. In comparison, a strictly homogeneous (uniform) distribution of 10% fibrosis resulted in a drifting rotor that quickly terminated after one rotation (see figure OS6A right panel and online movie 11). To further evaluate the role of homogeneity in the distribution of diffuse fibrosis in the control case we repeated the simulations utilizing geometry G-III of a normal PLA (see figure 6). Here we utilized normal ionic kinetics and 6% of diffuse fibrosis that was either homogeneously or randomly distributed. As
shown in panel B of Figure OS6, the results were very similar to those obtained with 10% of fibrosis. Namely, an anchored reentrant activity was observed when fibrosis was randomly distributed. In contrast, a drifting rotor that terminated rapidly was recorded when fibrosis was homogeneous (see also online movies 12 and 13).

Effects of SACs on AF dynamics

To investigate the possible role of atrial stretch in AF dynamics during HF, we implemented conditions for SACs kinetics utilizing geometry G-I, as well as HF kinetics and fibrosis distribution. The data presented in Figure OS7 compare results obtained in the absence (panel A) and the presence (panel B) of SACs. As shown in panel B, in the presence of SACs, the S1-S2 protocol initiated a reentrant source along a different cluster of fibrotic patches than when SACs were not present (panel A). With SACs, the reentry anchored more proximally to the PVO (see also online movie 14). The cause of this change in anchoring site was an increase in the wavelength (2.9 vs 2.2 mm) of the S1 impulse, which created an interaction between the S1 tail and the S2 wavefront resulting in unidirectional block close to the right PV ostium. Thus, in accordance with the experiments presented in figure 2B and 2C of the main article, the numerical data predict that the in the presence of PV ectopic activity stretch-related lengthening of wavelength might reproducibly initiate a reentrant source that would anchor to fibrotic patches at or near the PVO.

Effects of Heterocellular Electrical Coupling

In our simulations we have set some low degree of electrical coupling between fibroblasts and between fibroblasts and myocytes, in correspondence with some recent
evidence of such coupling. Additionally, we have assumed that the fibrotic patches stained in red in the histological slices are comprised of high-density fibroblasts. Still, in order to account for the possibility that these patches are merely comprised of insulating collagenous septa, we have repeated the simulations presented in figure 7 with geometry G-I without electrical coupling between fibroblasts and myocytes. The results presented in online movies 15-18 show some interesting differences in dynamics of propagation compared to figure 7 and the corresponding online movies 3-6. In the control condition, S1-S2 stimulation resulted in a sustained reentry whereas

References


FIGURE AND MOVIE LEGENDS

Figure OS1

A, B, C: Histological analysis of large PV ostial and central PLA areas in three different hearts (A, B and C). For each heart, a star indicates the areas that harbored the largest number of breakthroughs, as shown also in figure 2C of the main manuscript.

Figure OS2

Activation patterns and spatial distribution of endocardial breakthroughs sites at the PLA during atrial tachycardia and AF induced in the absence of ACh perfusion. A: Diagram of PLA with superposed dots representing breakthrough sites. Each breakthrough color corresponds to a given experiment (n=3). Grey disks represent PVO (abbreviations as in figure 1). B and C: AF episode phase movie snapshots of a repetitive breakthrough pattern located in the vicinity of the RSPV ostium (B) and of a reentrant activity anchored in the vicinity of the LSPV ostium. D: Corresponding RAA, LSPV and LAA electrograms.

Figure OS3

A: Transillumination experiment at the PLA-LAA junction. Top left, representative 50 ms isochronal map of epicardial activation during a single rotation of two counter-rotating waves emanating from an area of low amplitude. Bottom left, snapshot from amplitude movie showing reentrant waves emanating from low amplitude area. Right, the corresponding 1.5 sec time space plot, constructed along the white dashed line. Note transient episodes of christmas-tree like patterns that are enclosed by yellow dashed
boxes. One such box is magnified and reproduced as an inset in red dashed box. **B:** 3D simulation of a scroll wave in a cube of atrial tissue and corresponding time space plots. Upper panel, scroll wave filament is perpendicular to epicardial wall. Lower panel, the scroll wave filament is no longer in the field of view. Black dashed lines indicate the location of time space plot construction. **C:** Schematic showing the different time space plot patterns that would be observed in case of either an intramural U-shaped scroll filament or two transmural scroll waves of opposite chirality.

**Figure OS4**

Computer simulation in PLA transmural slices (geometry G-I), diffuse fibrosis distribution and HF kinetics (A) and patchy fibrosis distribution with control kinetics (B). **A.** Snapshots at several timeframes for cross-field stimulation (left) and pacing at a frequency of 20 Hz (right), as well as endocardial time-space plots (constructed for the segment marked by the red dashed line). **B.** Snapshots at several timeframes for cross-field stimulation (left) and pacing at a frequency of 6 Hz (right), as well as endocardial TSPs.

**Figure OS5**

Computer simulation implementing a peri-ostial (LIPV) area of a PLA transmural slice (geometry G-II) with patchy fibrosis distribution and control kinetics. Snapshots of electrical activity at timeframes 94, 122, 288, 359 and 423 ms. Reentry was initiated using S1-S2 stimulation and spatiotemporal endocardial activity is given as a TSP along the dashed red profile marked on the snapshot at 94 ms.
**Figure OS6**

Computer simulation implementing a random (left panels) or uniform (right panels) distribution of diffuse fibrosis in A: HF PLA geometry (G-I) and 10% of fibrosis, B: control PLA geometry (G-III) and 6% of fibrosis (see also online movies 11 and 12).

**Figure OS7**

Computer simulation implementing the geometry of the HF PLA (G-I) and patchy distribution of 10% fibrosis implementing SACs kinetics (panel B). For comparison, Panel A shows AF dynamics without SACs kinetics implementation.

**Online movie 1**

Reentrant activity anchored in the vicinity of the left superior pulmonary vein ostium during AF induced in the absence of ACh perfusion (see also figure OS2). The wavefront is depicted in blue-purple and the wavetail is shown in yellow.

**Online Movie 2**

Phase movie of PLA activation during AF in one representative normal heart showing very repetitive and centrally located endocardial breakthroughs. Same color code as in online movie 1.

**Online Movie 3**

Phase movie of PLA activation during AF in one representative HF heart showing very changing and peripherally located endocardial breakthroughs. Same color code than for online movie 1.
Online Movie 4
Numerical Simulation; 2D transmural PLA model. Geometry G-I. Diffuse heterogeneous 5% fibrosis distribution. Transmembrane voltage after S1-S2 stimulation. Voltages scale is normalized and shown from the lowest (dark blue) to the highest (red). Fibrosis architecture is artificially colored in red.

Online Movie 5
Numerical Simulation; 2D transmural PLA model. Geometry G-I. Patchy 10% fibrosis distribution. Transmembrane voltage after S1-S2 stimulation. Voltages scale is normalized and shown from the lowest (dark blue) to the highest (red). Fibrosis architecture is artificially colored in red.

Online Movie 6
Numerical Simulation; 2D transmural PLA model. Geometry G-I. Diffuse heterogeneous 5% fibrosis distribution. Transmembrane voltage movie during pacing stimulation at 20 Hz. Voltages scale is normalized and shown from the lowest (dark blue) to the highest (red). Fibrosis architecture is artificially colored in red.

Online Movie 7
Numerical Simulation; 2D transmural PLA model. Geometry G-I. Patchy 10% fibrosis distribution. Transmembrane voltage movie during pacing stimulation at 6 Hz. Voltages scale is normalized and shown from the lowest (dark blue) to the highest (red). Fibrosis architecture is artificially colored in red.
Online Movie 8
Numerical Simulation; 2D PLA periphery - LIPV ostium model. Geometry G-II. Patchy 10% fibrosis distribution. Transmembrane voltage movie after S1-S2 stimulation. Voltages scale is normalized and shown from the lowest (dark blue) to the highest (red). Fibrosis architecture is artificially colored in red.

Online Movie 9
Numerical Simulation; 2D PLA periphery - LIPV ostium model. Geometry G-II. Diffuse heterogeneous 5% fibrosis distribution. Transmembrane voltage movie after S1-S2 stimulation. Voltages scale is normalized and shown from the lowest (dark blue) to the highest (red). Fibrosis architecture is artificially colored in red.

Online Movie 10
Numerical Simulation; 2D transmural PLA model. Geometry G-I. Heterogeneous diffuse 10% fibrosis distribution. Transmembrane voltage movie after S1-S2 stimulation. Voltages scale is normalized and shown from the lowest (dark blue) to the highest (red). Fibrosis architecture is artificially colored in red.

Online Movie 11
Voltages scale is normalized and shown from the lowest (dark blue) to the highest (red). Fibrosis architecture is artificially colored in red.

**Online Movie 12**

Numerical Simulation; 2D transmural PLA model. Geometry G-III (control). Heterogeneous diffuse 6% fibrosis distribution. Transmembrane voltage movie after S1-S2 stimulation. Voltages scale is normalized and shown from the lowest (dark blue) to the highest (red). Fibrosis architecture is artificially colored in red.

**Online Movie 13**

Numerical Simulation; 2D transmural PLA model. Geometry G-III (control). Homogeneous diffuse 6% fibrosis distribution. Transmembrane voltage movie after S1-S2 stimulation. Voltages scale is normalized and shown from the lowest (dark blue) to the highest (red). Fibrosis architecture is artificially colored in red.

**Online Movie 14**

Numerical Simulation implementing SACs kinetics; 2D transmural PLA model. Geometry G-I. Patchy 10% fibrosis distribution. Transmembrane voltage movie after S1-S2 stimulation. Voltages scale is normalized and shown from the lowest (dark blue) to the highest (red). Fibrosis architecture is artificially colored in red.

**Online movie 15**
Numerical Simulation zero coupling between myocytes and non myocytes; 2D transmural PLA model. Geometry G-I. Diffuse heterogeneous 5% fibrosis distribution. Transmembrane voltage after S1-S2 stimulation. Voltages scale is normalized and shown from the lowest (dark blue) to the highest (red). Fibrosis architecture is artificially colored in red.

**Online movie 16**

Numerical Simulation zero coupling between myocytes and non myocytes; 2D transmural PLA model. Geometry G-I. Patchy 10% fibrosis distribution. Transmembrane voltage after S1-S2 stimulation. Voltages scale is normalized and shown from the lowest (dark blue) to the highest (red). Fibrosis architecture is artificially colored in red.

**Online movie 17**

Numerical Simulation zero coupling between myocytes and non myocytes; 2D transmural PLA model. Geometry G-I. Diffuse heterogeneous 5% fibrosis distribution. Transmembrane voltage movie during pacing stimulation at 20 Hz. Voltages scale is normalized and shown from the lowest (dark blue) to the highest (red). Fibrosis architecture is artificially colored in red.

**Online movie 18**

Numerical Simulation zero coupling between myocytes and non myocytes; 2D transmural PLA model. Geometry G-I. Patchy 10% fibrosis distribution. Transmembrane voltage movie during pacing stimulation at 6 Hz. Voltages scale is normalized and shown
from the lowest (dark blue) to the highest (red). Fibrosis architecture is artificially colored in red.
TABLE 1

Echocardiographic and characteristic data in baseline and failing hearts (n=7)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>22.9±0.9</td>
<td>30.9±0.7*</td>
</tr>
<tr>
<td>Baseline heart rate (beats/min)</td>
<td>85.9±2.7</td>
<td>150±10.5*</td>
</tr>
<tr>
<td>LA diameter (cm)</td>
<td>3.1±0.1</td>
<td>5.0±0.1*</td>
</tr>
<tr>
<td>LV end-diastolic diameter (cm)</td>
<td>3.5±0.2</td>
<td>4.6±0.2†</td>
</tr>
<tr>
<td>LV end-systolic diameter (cm)</td>
<td>2.2±0.2</td>
<td>3.7±0.3†</td>
</tr>
<tr>
<td>LV end-diastolic volume (ml)</td>
<td>54.2±8.2</td>
<td>99.6±13.7‡</td>
</tr>
<tr>
<td>LV end-systolic volume (ml)</td>
<td>17.7±3.0</td>
<td>63.5±11.6†</td>
</tr>
<tr>
<td>LV ejection fraction (%)</td>
<td>67.8±2.0</td>
<td>37.8±5.6*</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM. HF - heart failure; LA - left atrium; LV - left ventricular. * p<0.001, † p<0.01, ‡ p<0.05.
Figure OS1
Figure OS2
Figure OS4
Figure OS6
Figure OS7