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Electro-Anatomical Characterization of Atrial Microfibrosis in a Histologically Detailed Computer Model

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Abstract

Fibrosis is thought to play an important role in formation and maintenance of atrial fibrillation (AF). The propensity of fibrosis to increase AF vulnerability depends not only on its amount, its texture plays a crucial role as well. While the detection of fibrotic tissue patches in the atria with extracellular recordings is feasible based on the analysis of electrogram fractionation, as used in clinical practice to identify ablation targets, the classification of fibrotic texture is a more challenging problem. This study seeks to establish a method for the electro-anatomical characterization of the fibrotic textures based on the analysis of electrogram fractionation. The proposed method exploits the dependency of fractionation patterns on the incidence direction of wavefronts which differs significantly as a function of texture. A histologically detailed computer model of the right atrial isthmus was developed for testing the method. A stimulation protocol was conceived which generated various incidence directions for any given recording site where electrograms were computed. A classification method is derived then for discriminating three types of fibrosis, no fibrosis (control), diffuse and patchy fibrosis. Simulation results showed that electrogram fractionation and amplitudes and their dependency upon incidence direction allow a robust discrimination between different classes of fibrosis. Finally, to minimize the technical

effort, sensitivity analysis was performed to identify a minimum number of incidence directions required for robust classification.

Index Terms

Complex fractionated atrial electrograms; monodomain model; fibrosis classification

I. Introduction

Atrial conduction disturbances such as atrial fibrillation (AF) are among the most common tachyarrhythmias in adults, particularly in the elderly population [1]. AF is a complex multifactorial disease [2] that progresses with aging and the duration of AF itself, i.e., recurrent episodes of AF may lead to more severe forms of AF [3]. A significant amount of functional and structural remodeling takes place during disease progression which manifests in alterations of electrophysiological properties and propagation patterns [4], [5], [6], [7]. Among the numerous factors involved, the presence of fibrosis has been documented as a common feature in patients with AF [6].

Fibrosis has a profound impact upon electrical activation patterns at various size scales. At a microscopic size scale, fibrotic inlays disrupt the intracellular matrix, thus providing a substrate for structurally constrained conduction pathways through which electrical wavefronts stumble following complex zig-zag patterns. At a macroscopic size scale, these altered micro-propagations manifest as apparent conduction slowing, an important factor known to promote arrhythmias by shortening the effective wavelength [8]. Wavefront fractionation may also be observed at this size scale if fibrosis is severe enough to cause sufficiently large delays between adjacent depolarization events. Vulnerability to arrhythmias increases not only with the amount of fibrosis [9], its texture plays a crucial role as well. Textures are classified as interstitial, compact, patchy or diffuse [10] where patchy textures render tissue more vulnerable to arrhythmias than compact or diffuse textures [11].

The electrical signature of fibrosis in the extracellular space is an increase in electrogram complexity. While recordings in healthy well-coupled atrial tissue produce uniform atrial electrograms (UAEs), in the presence of fibrosis the tortuous conduction pathways induce complex fractionated atrial electrograms (CFAEs) [12]. CFAEs are characterized by multiple deflections which reflect the presence of multiple depolarization events in the vicinity of a recording site within a short time window. This causal link between fibrosis-mediated arrhythmias and its reflection in CFAEs led to a novel strategy in treating AF which relies upon targeting tissue under CFAE recording sites with ablation catheters [12], [13]. Although there are methods available to detect the presence of fibrosis based on electrogram fractionation [14], techniques exploiting fractionation indices to distinguish fibrotic textures have not been reported yet. In this study we aim to develop an electrogram-based method which allows the discrimination between different fibrotic textures. The proposed strategy exploits the dependency of fractionation patterns on the incidence direction of wavefronts at a given recording site. Using a histologically detailed *in silico* model of the rabbit right atrial isthmus we simulate wavefront propagation and extracellular electrograms in tissue areas which differed in their fibrotic texture. Unlike in experimental or clinical settings, tissue structure, source distribution and electrograms are available *in silico* at a high spatio-temporal resolution, thus allowing a thorough investigation of the correlation between texture and the CFAEs linked to it. A stimulation and recording protocol was conceived which generates wavefronts of various incidence directions at a given observation site. Based on differences in electrogram fractionation as a function of incidence direction a classification method is derived which is able to discriminate three

types of microstructures: (a) well coupled densely packed tissue; (b) uncoupled tissue with uncoupling structures oriented parallel to the cardiac fibers which can be classified as a patch texture; (c) uncoupled tissue with multidirectional arrangement of uncoupling structures which can be classified as diffuse texture.

II. Methods

A. Generating an Atrial Tissue Model

The inferior right atrial isthmus of an adult rabbit, the area between the inferior vena cava comprising the ramifications of the terminal crest and the tricuspidal valve, was selected as the region of interest for building an *in silico* tissue model since this region is considered a critical substrate for the genesis of intermittent conduction block, and as such is a clinical target for catheter ablation [15].

1) Histological Tissue Slices—A tissue sample containing the right atrial isthmus was excised, immediately fixed in 7% neutral buffered formaldehyde, dehydrated and embedded in paraffin. The paraffin block was serially sectioned parallel to the septal leaflet of the tricuspid valve using a microtome (Microm HM310, Thermo Fisher Scientific, Walldorf, Germany) with a sectioning width of 7 μm . Masson's trichrome staining protocol was used which labels connective tissue bluish-green, myocytes pinkish-red and nuclei blue-black. Cleft spaces remain unstained and appear white in the images.

2) Image Processing—Among a total of 144 serial sections, a representative slice covering a large portion of the region of interest was selected (see Fig. 1 A). This slice was then scanned (Scan Scope, Aperio, Vista CA, USA), and the resulted image was adjusted to $696 \times 1\,288$ pixels (pixel size 8 μm) in order to keep simulations computationally tractable.

The digitized image was segmented (Fig. 1 B) using k-means clustering [16] to discriminate the two domains relevant for building the computational model, that are electrically viable myocytes (red) and interstitial space composed of connective tissue (bluish-green) and intercellular clefts (white). ImageJ [17] was used to estimate amount, length and width of uncoupling structures (fibrosis and clefts). These structures appear as distinct objects, which were used to compute the following three metrics: 1) Area, i.e., sum of pixels of the object; 2) Length estimated by the major axis, which is the longest line connecting two arbitrary points in the boundary of an object; 3) Width, the longest line perpendicular to the major axis and connecting two arbitrary points of an object's boundary. Objects smaller than 8 pixels were eliminated, because the lengths and widths of such small objects would be meaningless. Finally, the color image was converted to grayscale to extract fiber orientations using an image intensity gradient method [18] (Fig. 1 C).

3) Mesh Generation—A finite element (FE) mesh was generated directly from the segmented histological image (Fig. 1 B). In this process, each pixel classified as being electrically excitable was represented by a hexahedral FE in the mesh whereas pixels classified as interstitial space or connective tissue were discarded. In a FE context, this imposes noflux boundary conditions along the interface between the two spaces which constrains conduction pathways, giving rise to complex activation patterns in the *in silico* model. However, in contrast to the *in vivo* situation, the *in silico* model is virtually 2D since it is based on a single histological section. Therefore, unlike in a full 3D tissue model which would include multiple serially sectioned histographs, depolarization wavefronts cannot circumvent isolating structures via conduction pathways which shunt an obstacle in the third dimension. To account for this 3D effect without registering multiple histographs, a second shunting layer was added. This layer was derived from the same histograph, but very fine-

scale microscopic details were omitted in the segmentation process. A threshold value was selected to segment small patches of connective tissue (< 1 mm) as myocytes while leaving larger anatomical barriers (e.g. separation between muscle bundles and large fibrotic patches) unchanged. Fiber orientations in the shunting layer were considered to be the same, however, eigenvalues of the conductivity tensor in Eq. 1, were reduced, being $20\times$ smaller. The inclusion of this shunting layer influences upon propagation patterns in two ways: i) Propagation across very small clefts is possible which would have been blocked otherwise; ii) While conduction across small clefts is possible, noticeable delays are introduced due to the slower conduction in the shunting layer, thus causing similar conduction delays as they would arise in 3D due to the longer conduction pathways required to propagate around an obstacle. Finally, each element of the mesh was classified as belonging to terminal crest (TC), pectinate muscle (PM), or vestibule (VE). In total, the model consisted of 2 695 299 nodes and 1 792 896 elements.

B. Type of Microfibrosis

To the best of our knowledge, there are no rigorous rules which would allow a quantitative discrimination of different types of fibrosis. In this study, the phenomenological classification method based on visual inspection of histological slices reported by Jong et al. [7] was considered. In their work, fibrosis is categorized according to its texture as being interstitial, compact, patchy, or diffuse fibrosis. The impact upon activation spread varies between these types. Compact fibrosis refers to tissue which is completely deprived of excitable myocytes, i.e. no propagation is possible there. Interstitial and patchy fibrosis mainly impair transverse conduction, but leave longitudinal conduction largely unaffected which results in zig-zag activation patterns. Diffuse fibrosis is characterized by a large number of rather fine fibrotic patches which do not influence significantly the activation pattern, but entail an overall conduction slowing. In this study, we aim to develop a method which allows to classify fibrotic tissue based on the the amount of uncoupling structures (collagen as well as interstitial clefts) and their spatial orientation with respect to the prevailing myocyte orientation. To develop and test this method, six representative regions of 1 mm^2 were selected (Fig. 2 A–B) by visual inspection in the chosen tissue slice which correlated with the following classifications:

- Well coupled densely packed tissue (WCDP) where propagation is uniform and continuous due to a low degree of uncoupling structures ($< 20\%$).
- Uncoupled tissue with uncoupling structures, $\sim 48\%$, oriented parallel to the cardiac fibers (UCPO) where propagation follows a zig-zag pattern caused by the presence of uncoupling inlays with average length $183\text{ }\mu\text{m}$ and $47\text{ }\mu\text{m}$ width similar to interstitial and patchy fibrosis.
- Uncoupled tissue, $\sim 70\%$, with multidirectional arrangement of uncoupling structures (UCMO) similar to diffuse fibrosis. Length and width could not be estimated in this case due to the diffuse nature of the fibrotic texture.

C. Governing Equations

Simulation of electrical activity was based on the monodomain equations [19]:

$$\nabla \cdot (\sigma \nabla V_m) = \beta I_m, \quad (1)$$

$$C_m \frac{\partial V_m}{\partial t} + I_{ion}(V_m, \eta) - I_{stim} = I_m, \quad (2)$$

$$\frac{\partial \eta}{\partial t} = g(V_m, \eta) \quad (3)$$

where σ is the intracellular conductivity tensor; V_m is the transmembrane voltage; γ is the membrane surface to cell volume ratio; I_m is the transmembrane current density; C_m is the membrane capacitance per unit area; I_{ion} is the density of the total ionic current flowing through the membrane channels, pumps and exchangers; and I_{stim} is the stimulus current density. I_{ion} depends on V_m as well as on a set of state variables η , which describes channel gating and ionic concentrations according to the vector-valued function $g(V_m, \eta)$. Spatial discretization relied on the FE method using hexahedral elements with piecewise linear weighting functions [20]. Equations were solved with an implicit-explicit scheme where the diffusion term is treated implicitly using a Crank-Nicolson scheme, and the reaction term explicitly using a Rush-Larsen technique [21], [22] with a time step of 10 μ s.

Cellular dynamics of rabbit atrial myocytes within the isthmus were described using the Aslanidi models [23]. Minor modifications were introduced to specialize myocytes models for the regions TC, PM and VE. The specialized cell models were assigned then to those elements in the FE mesh which made up the respective regions.

D. Simulation Protocols

1) Single-cell Simulations—Initial conditions were computed by pacing a single cell at a basic cycle length of 500 ms (2 Hz). Pacing was terminated when arriving at a stable limit cycle where differences in AP waveforms between subsequent beats were small ($< 1\%$). The state variables at the end of the protocol were stored for each type of myocyte, i.e. myocytes in the TC, PM or VE region, and used later as input for the tissue level simulations.

2) Tissue Simulations—Standard parameters for membrane capacitance $C_m = 1.0 \mu\text{F}/\text{cm}^2$, surface-to-volume ratio $\gamma = 0.14 \mu\text{m}^{-1}$ and conductivities longitudinal $\sigma_l = 0.174 \text{ S/m}$ and transverse $\sigma_t = 0.019 \text{ S/m}$ to the fiber direction were chosen based on experimental measurements [24]. The initial state of the tissue was defined by populating the model using state vectors as computed previously in single-cell pacing.

3) Circumferential Stimulation—In order to investigate the genesis of CFAEs and their dependence on the incidence direction of depolarization wavefronts, stimuli were applied at various sites around the selected tissue regions which we aimed to classify. As shown in Fig. 2 C, a stimulus current was injected at sites located along a virtual ring of 1 mm radius around the tissue region under investigation. In total, $s = 12$ simulations (one per stimulus site) were performed for each of the six tissue samples shown in Fig. 2 B.

E. Recovery of Extracellular Electrograms

Monodomain equations directly describe current flow only in the intracellular space, however, the electric potential field can be recovered by assuming that the tissue is immersed in a uniform volume conductor of infinite extent and conductivity σ_b . The relation used to recover extracellular electrograms is given by [25]:

$$\phi_e = \frac{1}{4\pi\sigma_b} \int \frac{\beta I_m}{\|\mathbf{r}\|} d\Omega \quad (4)$$

where $\mathbf{r}$ is the distance vector between source and field points. Using Eq. 1 the source term I_m is equivalent to $-\nabla \cdot (\sigma \nabla V_m)$ [26].

F. Data Analysis

Regular grids of recording sites (11×11 sites, $100 \mu\text{m}$ spacing) were defined to cover all six test regions in Fig. 2 B. Electrograms (ϕ_e) were recovered for each grid point at a distance of $60 \mu\text{m}$ above the tissue [27]. The amplitude ϕ_{eamp} of the biphasic unipolar electrogram, ϕ_e , calculated as the difference between its positive and negative peaks, as well as the fractionation index (FI), i.e. the number of negative peaks of the time derivative ϕ_e , were determined. Peaks of ϕ_e were calculated based upon an extrema-finding algorithm [28]. Deflections smaller than 10% of the signal peak-to-peak amplitude of ϕ_e were ignored.

1) Feature Extraction—In each of the six test regions the means of FI (μ_{FI}) and ϕ_{eamp} (μ_{amp}) of the $n = 121$ electrograms were computed for each of the $s = 12$ stimulus sites to extract the following metrics:

- a. m_{FI} : mean of μ_{FI} over all s stimulus sites.
- b. FI : range of μ_{FI} ($\Delta FI = \max_{n_s} \mu_{FI} - \min_{n_s} \mu_{FI}$, where $n_s = 1 \dots s$).
- c. amp : normalized range of μ_{amp} $amp = \mu_{amp} / m_{amp}$, where $\Delta amp = \max_{n_s} \mu_{amp} - \min_{n_s} \mu_{amp}$ and m_{amp} is the mean of μ_{amp} over all s stimulus sites.

2) Classification—A linear discriminant analysis (LDA) classifier [29] was employed which uses the electrogram-based metrics m_{FI} , FI and amp to classify three types of microstructure, i.e. WCDP, UCPO, and UCMO. Cross validation was done using the leave-one-out method [30]. Evaluation criterion was the classification accuracy [31], i.e. the fraction of correctly classified samples for each class (acc_1 , acc_2 , and acc_3) as well as the overall accuracy acc_{tot} .

3) Sensitivity Analysis—The robustness of the proposed classification method with respect to the number of incidence directions, s , as well as recording sites, n , which varied as a function of the electrode grid density d , was assessed. Since the choices of s and n are restricted by the technical design of experimental and clinical mapping systems, this sensitivity analysis is important to determine the minimum requirements for achieving robust classification with a desired accuracy. Accordingly, s was reduced from 12 to 6 and 3 incidence directions, respectively, and grid densities were varied in the range $d = 200, 300, 400$ and $600 \mu\text{m}$, which yielded between 4 and 121 electrogram recording sites. In the case of $s = 6$, features were computed for stimulus positions $0^\circ, 60^\circ, 120^\circ, 180^\circ, 240^\circ$ and 300° . Subsequently, stimulus sites were rotated by 30° along the ring centered around the tissue region under investigation, and all features were recomputed. Thus, e.g. for $s = 3$, three feature sets were obtained. Similarly, for each grid density grids were shifted by half of the grid spacing along the x and y axes. For instance, for $d = 200 \mu\text{m}$ the recording grid was shifted along x and y directions by $[x \ y]^T = [100 \ 0] \mu\text{m}$, $[x \ y]^T = [0 \ 100] \mu\text{m}$, and $[x \ y]^T = [100 \ 100] \mu\text{m}$. Thus, in the case $d = 200 \mu\text{m}$, four feature sets were obtained.

G. Computational Aspects

Monodomain equations were solved using the Cardiac Arrhythmia Research package (CARP). Details of the underlying numerical methods have been described elsewhere [22], [32]. This work made use of the facilities of HECToR, the UK's national high-performance computing service, which is provided by UoE HPCx Ltd at the University of Edinburgh, Cray Inc and NAG Ltd, and funded by the Office of Science and Technology through EPSRC's High End Computing Programme.

III. Results

A. Directional Dependency of Fractionation

Fig. 3 presents extracellular electrogram traces, ϕ_e , and their time derivatives, ϕ_e' , taken from the center of the recording grids in WCDP(1), UCPO(1) and UCMO(1). The signals were obtained after simulating the circumferential protocol in which stimuli were applied at $s = 12$ sites, as illustrated in Fig. 2 C, to initiate activation sequences of varying incidence direction. Note the absence of fractionated electrograms among the signals obtained in the WCDP case meaning that uniform conduction is dominant and independent of incidence direction of wavefronts. Representative examples illustrating the dependency of FI and ϕ_{eamp} upon incidence direction can be seen for UCPO in Fig. 3 (middle panel). An uniform atrial electrogram (UAE) with $FI = 1$ was observed when delivering a stimulus at 30° , while a complex electrogram with a higher FI and significantly smaller amplitudes in both ϕ_e and ϕ_e' arose for the stimulus delivered at 330° . Signals obtained in the UCMO case were characterized by high FI and small ϕ_{eamp} . Note that the electrogram computed after stimulating at 90° was marked as being an UAE despite the presence of a second and smaller peak. This is because either the amplitude of the second peak is smaller than 10% of the peak-to-peak amplitude of ϕ_e or the temporal coincidence of the two activations was too close (see Sect. II-F and reference [28]).

B. Feature Extraction

In all six sample regions shown in Fig. 2B, stimuli were applied at $s = 12$ sites following the circumferential protocol (Fig. 2 C). Electrograms were recovered at $n = 121$ recording sites, for all 12 incidence directions. For each ϕ_e trace, FIs were computed from the temporal derivative ϕ_e' as well as signal magnitudes ϕ_{eamp} directly from ϕ_e . For all tissue patches under investigation, the means over all n recording sites of FI, μ_{FI} , and of ϕ_{eamp} , μ_{amp} , were plotted as a function of incidence direction. Results are summarized in Fig. 4.

C. Classification

Based on visual inspection of the underlying histology selected tissue patches were classified either as WCDP, UCPO or UCMO. This histology-based classification served as a reference for testing the proposed electrogram-based classification method which relied on the features m_{FI} , FI and μ_{amp} extracted from unipolar extracellular recordings. Classification results are illustrated in Fig. 5 for the cases with the smallest and largest number of sample sets. That is, 6 sample sets computed with $s = 12$ incidence directions and sites $n = 121$ recording sites (Fig. 5A), and 648 sample sets computed with $s = 3$ and $n = 4$ (Fig. 5B), respectively. Quantitatively, the mean feature coordinates of the three types of microstructure separate quite well. Note that even in the case with less incidence directions and recording sites, the respective sample clouds do not overlap significantly, suggesting that accurate separation of tissue types based on features extracted from extracellular electrograms is possible.

D. Sensitivity Analysis

The cost of classifying a single tissue patch depends on the number of incidence directions s and the number of recording sites n . In the extreme case where $s = 12$ incidence directions and $n = 121$ recordings were used, these costs are high. To determine the minimum requirements in terms of number of incidence directions s and extracellular electrograms n for reliable classification a sensitivity analysis was performed. For this sake, the same analysis was repeated using reduced numbers s and n (reducing thus the grid density d). Results are summarized in Tabs. I, II, and III where each individual table refers to a given number s of incidence directions. Classification accuracies for each of the three classes (acc_1

for WCDP, acc_2 for UCPO, and acc_3 for UCMO) as well as the overall accuracy acc_{tot} are given. In Fig. 6 the sensitivity results are illustrated where overall classification accuracy is plotted as a function of incidence direction and grid density s/d . To classify with accuracies better than 90%, a grid spacing of $400\ \mu m$ (equivalent to 9 recording sites) or finer, and 6 or more incidence directions are required.

IV. Discussion

Endocardially recorded electrograms and their interpretation form the diagnostic basis of minimally invasive therapies for atrial arrhythmias which rely on modifying conduction pathways [13]. In recent years, atrial fibrosis has been identified as an important target for catheter ablation [12], [13]. The signature of fibrosis in extracellular space is the formation of CFAEs which is due to the presence of multiple depolarization events in the vicinity of a recording site. CFAEs consist of two or more discrete deflections, arising in areas where conduction is impaired due to the presence of connective tissue and fiber disarray [33], [34]. The link between CFAEs and cardiac microstructure has been previously investigated in detail using microscopic size scale computational models of 2D tissue sheets [14], [35], [36]. However, several studies suggest that not only amount of fibrosis, but also its texture is a key factor [7], [37], [38].

While this causal link between CFAEs and fibrosis is firmly established, and the presence of CFAEs is used as a surrogate indicator for the presence of fibrosis at the recording site in the clinic, the characterization of the type of fibrosis based on the interpretation of CFAEs has not been attempted yet. The method proposed in this study exploits the dependency of electrogram fractionation on the incidence direction of depolarization wavefronts relative to the topological structure of fibrotic inlays [35], [36]. In this study we established a histologically detailed computer model of the rabbit right atrial isthmus, which accounts for the complex spatial organization of microfibrosis [7], [15], [39]. To demonstrate the feasibility of CFAE-based fibrosis classification, different regions were selected in the model which could be classified as WCDP, UCPO or UCMO by visual inspection of the underlying histology. Such visual classification of fibrotic texture, which is not feasible in any experimental or clinical context, served as a ground truth against which the proposed CFAE-based classification method was compared. It is of importance that the proposed method is purely based on electrogram traces which can be assumed to be readily available in any applied scenario. Using a stimulation protocol which induces wavefronts of varying incidence directions at the tissue patch under investigation, CFAEs were computed and features were extracted to characterize the variation of signal amplitude and degree of fractionation as a function of incidence direction. Results suggest that the separability with the proposed method is quite good, allowing a robust classification of the type of fibrosis.

A. Choice of Classification Features

Whether CFAEs manifests in extracellularly recorded electrograms or not is governed by two key factors which are, on one hand, the presence and spatial organization of uncoupling structures, and, on the other hand, the curvature and incidence direction of propagating wavefronts. Various methods for detecting CFAEs with extracellular electrogram recordings have been reported which derived fractionation indices from the unipolar electrogram, ϕ_e [40], or its temporal derivative, ϕ_e [28], or from bipolar electrograms [34]. While these methods may allow to detect the presence of fibrosis, they cannot be used to characterize its texture. Independently of which type of fractionation index is used, CFAEs will only manifest in those cases where incidence direction and electrically isolating fibrotic structures are non-parallel. Otherwise even progressive patchy fibrosis may remain undetected without any fractionation signature in recorded electrograms, since the conduction delays induced by the isolating structures are not sufficiently large along strands of myocytes aligned with the

isolating structures. This dependency on incidence direction, as observed in previous experimental and computer simulation studies [36], [41], provides the rationale for our choice of classification features. m_{FI} is a measure of how FI changes as a function of wavefront incidence direction which can be steered by varying the stimulus site relative to the observation site. In non-fibrotic tissue, m_{FI} is expected to be 1, regardless of incidence direction. In cases of patchy fibrosis such as the UCPO patches, m_{FI} is supposed to vary whereas in cases of diffuse fibrosis such as the UCMO patches, m_{FI} is always present independently of the incidence direction. FI aims to quantify this variation in FI as a function of wavefront incidence direction. That is, FI is expected to be small in WCDP as well as in UCMO tissue, only in UCPO tissue FI is expected to be large due to the strong dependency of fractionation on incidence direction.

Additional information on tissue structure is gained from taking into account that electrogram amplitudes, as shown in Fig. 4 (WCDP lower panel), are influenced by tissue anisotropy. Incidence directions where wavefronts propagate in a direction transverse to the prevailing myocyte orientation, are characterized by smaller amplitudes relative to those propagating in a longitudinal direction [42]. Thus, amp , a metric for the amplitude change of signals as a function of incidence direction, was chosen as an additional feature. In absence of anisotropy, amp becomes independent of incidence direction. This might be the case with diffuse fibrotic patterns such as the UCMO cases in this study. With such textures, connective inlays compartmentalize the tissue in such a way that the fibrotic networks govern the speed of propagation, and not the orientation of myocytes. This notion is supported by the UCMO case in Fig. 4 (UCMO lower panel) where amp is small.

Additional features such as, for instance, the local activation time dispersion within fractionated electrograms as well as the selection of appropriate features by means of ranking algorithms (e.g. [43]) might improve the classification accuracy.

B. Choice of Classifier

A rather simple LDA classifier was chosen since scatter plots of the three-dimensional feature space suggested a good separability by hyperplanes. While other more elaborate classifiers may result in a better classification performance we refrained from investigating since we deemed the accuracy achieved with the LDA method to be appropriate. The leave-one-out method for cross validation was chosen, because in the case of $s = 12$ and $d = 100 \mu\text{m}$, only two samples per class (6 samples in total) were available. Other cross validation methods would presumably deselect all samples of a certain class from the set of training data or test data, respectively, and thus lead to a biased result of classification accuracy.

C. Number of Incidence Directions and Recording Sites

In any experimental or clinical setting, the number of available stimulation and recording sites is restricted, either due to the technical limitations of mapping systems or the time frames available for completing an experiment or an intervention. Therefore it is important to establish as to which degree the classification accuracy depends on the number of stimulus and recording sites. Tabs. I, II, and III reveal that the number of stimulus sites is the key factor for achieving a high classification accuracy. Reducing the number of incidence directions had a stronger impact on classification accuracy as compared to reducing grid density of the recording array. The range of values chosen for number of stimulus sites, grid size, and number of recording electrodes is considered to be technically feasible for *in vitro* experiments. Arrays with 32 electrodes with interelectrode spacing of $300 \mu\text{m}$ are available (*FlexMEA36; Multichannel Systems*, Reutlingen, Germany). Electrode arrays for measuring multiple unipolar electrograms as well as stimulating tissue at different sites have been used in *in vivo* experiments [44]. However, the requirements on inter-electrode distance and

number of electrodes determined in this work, would require the development of a new dedicated sensor.

D. Spatio-temporal Scales in CFAE Genesis

CFAEs arise at all scales of structural organization, ranging from microscopic structures of a few tens of μm , which induce conduction delays of only a few tens of μs , to the macroscopic scale where large fibrotic patches of a few millimeters induce delays of some tens of milliseconds. The size scales of CFAEs which can be detected with a given sensor depend on the size of electrode pads and sampling frequency. In *in vitro* experiments, ultra small electrodes with pad sizes of $10\text{ }\mu\text{m}$ are used with sampling frequencies up to 200 kHz , allowing to detect conduction delays between wavefronts down to $10\text{ }\mu\text{s}$ [27]. Clinically, much larger catheter electrodes and low bandwidth sampling blur and filter any information at the microscopic size scale which would allow the separation of depolarization events. While fibrotic structures which induce CFAEs observable at the macroscopic size scale are likely to be a relevant factor in atrial arrhythmogenesis, this is less clear with microfibrosis, as investigated in this study. In pathological cases where conduction is slowed and action potential duration is shortened to a significant degree, microfibrosis which would not manifest in CFAEs as defined in the clinic, may play a role in initiation as well as maintenance of atrial arrhythmias. For instance, the dissociation between endocardial and epicardial layers in the atria is discussed as a potential factor since the size scale of fibrotic structures in the transmural direction is, due to the thinness of the atria, in the sub-millimeter range [45].

E. Fractionated Electrograms During Atrial Arrhythmias

In this work, we proposed a method for characterizing distinct fibrotic textures based uniquely on changes in electrogram morphology in response to different incidence directions of wavefronts. We demonstrate that fractionation is not a local tissue feature, it depends, to a large degree, on incidence direction as well. A stimulation protocol was conceived to probe systematically various incidence directions, i.e., not only one direction as in sinus rhythm, using ideal infinitely small virtual electrode pads. In contrast to the circumferential protocol, during atrial arrhythmias such as AF the presence of multiple wavelets impedes to determine incidence direction with certainty. Further, during AF additional fractionation may be added to a signal due to contributions of wavelets traversing adjacent tissue which is unrelated to the fibrotic state at given recording site. Furthermore, the curvature of small wavelets is more pronounced as compared to sinus rhythm, thus one can expect more complex wavefront-fibrosis interactions. The link between ablation-targeting CFAEs and sinus rhythm fractionation has been investigated in a recent clinical study by Saghy *et al.* [46]. The authors showed that a non-fragmented electrogram recorded during sinus rhythm in a patient with AF turned into a fractionated electrogram during coronary sinus pacing. Thus, demonstrating the dependency of electrogram fractionation on incidence direction in the clinical context. As opposed to their study, which used a circular clinical mapping catheter with a fairly large size and limited far-field suppression, in our work, unipolar point-like electrograms were computed which closely match those measured using high resolution mapping systems [27], [47]. That is, far-field effects or by-stander activations can be ruled out. Indeed, Jacquemet and Henriquez [14] presented a computational study in which CFAEs recorded by electrodes with larger tips were more asymmetric and had smaller amplitudes when compared to punctual electrodes due to spatial averaging effects present in the former. The differentiation between normal sinus rhythm fractionation and ablation-targeting fractionation due to an underlying arrhythmia is not within the scope of our study. Here we pursued a simpler first step towards this goal, namely whether or not the proposed method allows to classify fibrotic tissue, assuming an ideal infinitely small electrode pad. Therefore, an extrapolation of our findings to support any conclusions on arrhythmogenicity

or to clinical scenarios, where large catheter tips are used and the incidence direction of wavefronts can not be determined with certainty, would require an entirely new study.

F. Limitations

The 2D computer model used in this work is based on a single histological image of the rabbit right atrial isthmus. While it accounts for structural heterogeneities due to microfibrosis and interstitial clefts, current flow is confined to 2D and 3D effects remain unaccounted for. A shunting layer was used to alleviate this limitation, mimicking 3D current flow around otherwise isolating tissue structures. Reduced conductivity in the shunting layer led to slower conduction, causing conduction delays across clefts as they would arise in 3D due to the longer 3D conduction pathways. The choice of only six tissue patches of 1 mm² stems from the fact that tissue samples were obtained from an adult, but not senescent rabbit. Thus, fibrotic tissue is found, but not to an abundant degree. For testing the proposed method only 2 samples per class, 6 samples in total, were chosen which represent clear examples of the respective class. Therefore, the obtained classification accuracies might be too optimistic. Finally, only ideal, infinitely small unipolar electrodes were considered. While electrodes with very small pad sized down to 10 µm, which come close to this ideal, are technically feasible and have been used in experimental studies [27], electrode sizes routinely applied in experiments or in the clinic are larger. Since electrode sizes strongly influence electrogram complexity [14] the proposed classification method may require adjustments when used with larger electrode pads.

G. Conclusions

In this study, computational modeling was used to characterize different types of spatial organization of uncoupling structures, namely microfibrosis and interstitial clefts during paced rhythm. The findings support the hypothesis that microstructural organization of fibrosis and the direction of the electrical wavefront are linked to CFAEs. Changes in electrogram morphology suggest that changing the site of stimulus allows to distinguish among different types of fractionation patterns of propagation, namely those arising from uncoupling structures oriented parallel to the cardiac fibers (patchy texture) from others associated with multidirectional arrangement of uncoupling structures (diffuse texture). Electrogram fractionation and amplitude were shown to be useful measurements to characterize spatial organization of microfibrosis. The ability of distinguishing among different types of fibrosis represents an important step towards understanding the mechanisms behind AF. From the clinical perspective, our findings could be helpful to elucidate whether the presence of CFAEs represents reliable targets for catheter ablation.

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Fig. 1.

(A) Trichrome stained histological image of the right atrial isthmus showing myocytes (red), connective tissue (bluish-green), and interstitial clefts (white). Location of the anatomical landmarks tricuspid valve (TV) and the inferior caval vein (ICV) is indicated. (B) Segmented image used for construction of the computer model where anatomical regions are shown with different gray scales, i.e. terminal crest (TC) in black, pectinate muscles (PMs) in dark gray, and vestibule (VE) in light gray. (C) Computed fiber orientation.

Fig. 2.

(A) Histological slice used to construct the computer model. Chosen representative tissue patches for the three types of microstructure are marked with squares. (B) Zoomed view on selected tissue patches. From top to bottom: well coupled densely packed tissue (WCDP), uncoupled tissue with uncoupling structures oriented parallel to the cardiac fibers (UCPO), and uncoupled tissue with multidirectional arrangement of uncoupling structures (UCMO). (C) Schematic representation of circumferential stimulus arrangement with $s = 12$ stimuli (from 0° to 330°). Sites of stimulation are demarcated in yellow.

Fig. 3.

Dependency of electrogram morphology upon incidence direction. Electrograms ϕ_e and time derivatives $\dot{\phi}_e$ computed after applying the circumferential protocol to tissue patches WCDP(1), UCPO (1) and UCMO(1) in Fig. 2. Signals were taken from the center (yellow dot) of the recording grid as illustrated for WCDP(1). Color coding refers to the degree of fractionation $FI = 1$ (black), $FI = 2$ (cyan) and $FI > 2$ (orange).

Fig. 4.

Dependency of μ_{FI} and μ_{amp} on incidence direction (from 0° to 330°) for all six different types of tissue microstructure WCDP(1)–(2), UCPO(1)–(2) and UCMO(1)–(2) shown in Fig. 2 B. μ_{FI} and μ_{amp} shown were computed for the tissue samples marked with (1) in the left panel of Fig. 2 B.

Fig. 5. Feature space. 2D plots showing sample sets within a standard deviation around the means of m_{FI} , FI , and amp for: (A) Smallest number of sample sets (6) computed with $s = 12$ incidence directions and $d = 100 \mu m$ grid density. (B) Largest number of sample sets (648) computed with $s = 3$ and $d = 600 \mu m$. The centers μ_{FI} , μ_{amp} and the mean FI are indicated by a +.

Fig. 6. Sensitivity analysis. Overall accuracy acc_{tot} plotted as a function of number of incidence directions s and recording grid density d .

TABLE I

Classification results for $s = 12$ Incidence directions. Features were obtained from n electrograms recorded in a grid with varying length d yielding the number of sample sets (See Sect. II-F3). Classification accuracies are given for each of the three classes (acc_1 for WCDP, acc_2 for UCPO and acc_3 for UCMO) as well as the overall accuracy acc_{tot} .

d μm	Recording sites (n)	Sample sets	acc_1	acc_2	acc_3	acc_{tot}
100	121	6	1.00	1.00	1.00	1.00
200	36	24	1.00	1.00	1.00	1.00
300	16	54	1.00	1.00	1.00	1.00
400	9	96	0.97	0.97	1.00	0.98
600	4	216	0.93	0.91	0.99	0.94

TABLE II

Classification results for $s = 6$.

d μm	Recording sites (n)	Sample sets	acc_1	acc_2	acc_3	acc_{tot}
100	121	12	1.00	1.00	1.00	1.00
200	36	48	1.00	1.00	1.00	1.00
300	16	108	0.96	0.96	1.00	0.97
400	9	192	0.98	0.98	1.00	0.98
600	4	432	0.90	0.87	0.99	0.92

TABLE III

Classification results for $s = 3$.

d μm	Recording sites (n)	Sample sets	acc_1	acc_2	acc_3	acc_{tot}
100	121	18	1.00	0.83	0.83	0.89
200	36	72	1.00	0.87	0.88	0.92
300	16	162	1.00	0.87	0.89	0.92
400	9	288	0.99	0.84	0.87	0.90
600	4	648	0.90	0.70	0.85	0.82