Published in final edited form as:

Conf Proc IEEE Eng Med Biol Soc. 2010 ; 2010: 2541–2544. doi:10.1109/IEMBS.2010.5626870.

A 2D-Computer Model of Atrial Tissue Based on Histograms Describes the Electro-Anatomical Impact of Microstructure on Endocardiac Potentials and Electric Near-Fields

Fernando O. Campos,

Institute of Biophysics, Medical University of Graz, Harrachgasse 21/IV, A-8010 Graz, Austria

Thomas Wiener,

Institute of Biophysics, Medical University of Graz, Harrachgasse 21/IV, A-8010 Graz, Austria

Anton J. Prassl,

Institute of Biophysics, Medical University of Graz, Harrachgasse 21/IV, A-8010 Graz, Austria

Helmut Ahammer,

Institute of Biophysics, Medical University of Graz, Harrachgasse 21/IV, A-8010 Graz, Austria

Gernot Plank,

Institute of Biophysics, Medical University of Graz, Harrachgasse 21/IV, A-8010 Graz, Austria

Rodrigo Weber dos Santos,

Department of Computer Science, Universidade Federal de Juiz de Fora, Campus Universitário, 36036-330 Juiz de Fora - MG, Brazil. rodrigo.weber@ufjf.edu.br

Damián Sánchez-Quintana, and

Departamento de Anatomía, Biología Celular y Zoología, Universidad de Extremadura, Avenida de la Universidad, 10003 Cáceres, Spain. damians@unex.es

Ernst Hofer

Institute of Biophysics, Medical University of Graz, Harrachgasse 21/IV, A-8010 Graz, Austria. ernst.hofer@medunigraz.at

Abstract

In experiments with cardiac tissue, local conduction is described by waveform analysis of the derivative of the extracellular potential $\dot{\Phi}_e$ and by the loop morphology of the near-field strength E (the components of the electric field parallel and very close to the tissue surface). The question arises whether the features of these signals can be used to quantify the degree of fibrosis in the heart. A computer model allows us to study the behavior of electric signals at the endocardium with respect to known configurations of microstructure which can not be detected during the electrophysiological experiments. This work presents a 2D-computer model with sub-cellular resolution of atrial micro-conduction in the rabbit heart. It is based on the monodomain equations and digitized histograms from tissue slices obtained post-experimentum. It could be shown that excitation spread in densely coupled regions produces uniform and anisotropic conduction. In contrast, zones with parallel fibers separated by uncoupling interstitial space or connective tissue may show uniform or complex signals depending on pacing site. These results suggest that the analysis of $\dot{\Phi}_e$ and E combined with multi-site pacing could be used to characterize the type and the size of fibrosis.

I. INTRODUCTION

Atrial fibrillation and flutter are to some degree associated with the change of tissue structure during aging [1]. Connective tissue is increasingly found with aging, which separates groups of myocardial fibers electrically. At the endocardial surface this alters conduction from uniform towards complex and turns smooth biphasic and uniform atrial electrograms (UAE) into complex fractionated atrial electrograms (CFAE). Heterogeneity in tissue ranging from some millimeters down to cellular and subcellular level can be found in the atrium. The electro-anatomical characterization of such substrates by means of multisite recording techniques of the extracellular potential Φ_e , its derivative $\dot{\Phi}_e$ and the cardiac near-field (CNF) strength $\mathbf{E}$ is the focus of experimental work in our lab with isolated atrial heart tissue from Rabbits. $\mathbf{E}$ describes a vector-loop which varies in morphology from a straight line to an “open” loop similar to those seen in a vector cardiogram [2]. The peak vector of $\mathbf{E}$ points in the opposite direction to the local propagation and is oriented perpendicular to the isochrones of activation. Its morphology changes with curvature of the wavefront as well as with the probe orientation [2]. In electrophysiological experiments, smooth biphasic electrograms as well as complex electrograms with different degree of fractionation can be found exploring the endocardial area of the cavotricuspidal isthmus [3] comprising the Crista Terminalis (CT), Pectinate muscles (PMs) and the Vestibulum (see Figure 1a). Since microstructure is not visible during the in-vitro experiment, details of relationship between connective tissue structure and signal morphology remain hidden. In this work we describe a 2D-model of this region of interest (ROI) derived from a digitized histograph produced post-experimentum (Figure 1b). The structural and functional model comprises local fiber direction and distinguishes between myocytes, connective tissue and intercellular space. This virtual tissue can be paced and the answer in terms of activation sequence and signals can be computed at any given site with ultra high spatio-temporal resolution. In contrast to experimental data, the signals are free of noise and the relationship to the local structure can be seen from the underlying micrograph. We have chosen regions representing two types of microstructure: a. densely packed and electrically well coupled tissue and b. uncoupled tissue with interstitial clefts oriented parallel to the fibers. This work describes the computer model, the resulting activation patterns at a microscopic scale and the behavior of signals $\dot{\Phi}_e$ and $\mathbf{E}$ in the ROI when changing the pacing site e.g. the direction of the local wavefront of excitation.

II. METHODS

A. Atrial Tissue Slice and Determination of the ROI

The atrial tissue was fixed with a mixture of paraformaldehyde-glutaraldehyde, 10% formaline solution and embedded in paraffin sections of 10 μm thickness. Although it is known that formalin fixation causes a shrinkage of the tissue sample (5 - 10% of the total volume), this choice was made since it does not affect neither the myocardial architecture nor the intramyocardial connective tissue. The staining (Massons Trichrome) resulted in a coloring of muscle cells in red, of collagen and Reticulin fibers in cyan and of intercellular clefts in white. Micrographs were then digitized (San Scope, Aperio, Vista CA, USA) in SVS file format (12.3 mm $\times$ 9.6 mm with 50 483 $\times$ 41 280 pixels). For building the computer model the digital image was cropped and reduced in resolution to (5.9 mm $\times$ 3.8 mm with 670 $\times$ 458 pixels) in order to keep the simulations tractable. After visual inspection of the tissue slice, two different subregions were chosen with different characteristics of interstitial connective tissue. In the subregion A1 cells are tightly coupled with few and very thin intercellular clefts and connective tissue. The subregion A2 shows dense fibrosis characterized by connective tissue and intercellular clefts of multiple cell length oriented parallel to the axis of fibers. The two subregions are demarcated in Figure 1b.

B. Simulation Protocols

The digitized image was segmented in the hue, saturation and lightness (HSL) color space. Proper thresholds were set in order to distinguish myocardium from connective tissue and intercellular clefts [4]. The membrane kinetics in atrial myocytes (Rabbit) are modeled by a set of nonlinear equations described by the Lindblad *et al.* model [5], whereas connective tissue as well as intercellular clefts were removed from the computational grid. The preferential pathway for activation, i.e. the local direction of fibers, was obtained using an image intensity gradient method [6]. In total, 348 670 nodes with a space discretization of $9 \mu\text{m}$ are required to capture microstructural details found in a $5.9 \times 3.8 \text{ mm}^2$ area of tissue. This model reveals the complexity of activation patterns caused by the microstructure but does not reflect the shunting effects of deeper layers of tissue. Therefore, a second layer of cardiac tissue was added based on the same histograph, but neglecting microscopic details and introducing isotropy. The coupling between the two 2D-sheets was set with a higher resistance compared to the upper layer. Due to this shunting layer, activation was able to propagate into local regions that were disconnected in the 2D-model. 557 637 nodes connecting 319 704 hexahedral elements were used to construct this 3D approach.

C. Computational Methods

In order to investigate the occurrence of fractionated electrograms and their dependence on the direction of the depolarization wavefront, three simulations were performed. A stimulus current was applied at three different sites (Figure 1b): STL1, close to the sinoatrial node (SAN) causing an antegrade propagation along the CT; STL2, located distal to the SAN in order to produce retrograde conduction in the CT; and STL3, placed in a PM producing a retrograde conduction in it. All simulations were carried out for 30 ms computing only the depolarization phase after the stimulus. The simulations of the excitation spread were performed with the Cardiac Arrhythmia Research Package (CARP) [7] using the monodomain equations [8].

D. Signal Analysis

Although the monodomain equations describe only current flow in the intracellular medium of the cardiac tissue, the extracellular potentials can be recovered using:

$$\Phi_e(x_F, t) = \frac{1}{4\pi\sigma_e} \int \frac{I_m(x_T, t)}{|D_{TF}|} d\Gamma_T \quad (1)$$

where x_F is the point of observation, x_T the source point, $|D_{TF}|$ the distance between x_F and x_T , σ_e the conductivity of the homogeneous volume conductor, and Γ_T is the surface area of the tissue [2].

Two regular areas containing 56 and 76 points spaced by $126 \mu\text{m}$ were taken to represent subregions A1 and A2, respectively (Figure 1b). Each of these points represent the center of a square CNF electrode with $50 \mu\text{m}$ lateral length. Electrograms were computed in the middle and at the vertices of each electrode. All signals were computed $60 \mu\text{m}$ above the tissue surface to match our experimental system [9]. The time derivative of the extracellular signals ($\dot{\Phi}_e$) was computed. As a measure of complexity, the fractionation indices (FI), i.e. the number of negative peaks of $\dot{\Phi}_e$ were calculated by an extrema-finding algorithm. Deflections smaller than 10% of the signal peak-to-peak amplitude of $\dot{\Phi}_e$ were ignored. The near field strength $\mathbf{E} = [E_x \ E_y]^T$ was computed according to [2]. Peak values $|\mathbf{E}|_{pp}$ and $\dot{\Phi}_{ep}$ were given in percentage of the respective maximum value in each subregion.

E. Computing Environment

The simulations of the electrical activity were performed on an Intel Xeon 2.33 GHz dual-core processor, 4 GB of RAM and 800 GB of hard disk running a 64-bit Kubuntu Linux system. Each simulation run took 10 h of execution time. The results, i.e. the set of transmembrane potentials were stored with sampling intervals of 20 μ s. The output file contains 3.1 GB of data. In order to recover Φ_e around all recording sites, an weighted sum of the currents over all nodes in the mesh has to be computed. This post-processing step took 1 h to complete.

III. RESULTS

Figure 2 shows how the wavefronts initiated from three different pacing sites propagate into subregions A1 and A2. Note that regardless the origin of the activation, the wavefront enters A1 always with a low curvature (Figure 2 left hand side). It can be seen that conduction is longitudinal antegrade in STL1 and longitudinal retrograde in STL2 whereas pacing at STL3 induces transverse conduction in A1. This is also demonstrated in the vector-loops of $\mathbf{E}$ depicted in Figure 3, whose peaks are pointing in the opposite direction to local propagation spread. Table I and Figure 3 (right column) show that conduction in A1 is quite uniform with dominance of UAE (with at least 54 out of 56 recording sites) due to its electrically well coupled structure. $\dot{\Phi}_{ep}$ and $|\mathbf{E}|_{pp}$ are similar during antegrade and retrograde longitudinal conduction (STL1 and STL2) whereas during transverse conduction both parameters are decreased (STL3).

Regarding A2, we detect considerable delays of the wavefronts among three muscle bundles depicted in Figure 2d and Figure 2f (pacing in STL1 and STL3 respectively), but not when the activation was started in STL2 (Figure 2e). In STL1, this is expressed by CFAE as shown in Figure 4a. The number of fractionated electrograms (N2 and N3+) account for 72% of the signals (Table II) and $\dot{\Phi}_{ep}$ as well as $|\mathbf{E}|_{pp}$ are smaller when compared with the other two pacing sites. However, when the wavefront starts from STL2, the profile changes and the UAE dominates again (Figure 4b and Table II). STL2 produces less complex vector-loops and $\dot{\Phi}_{ep}$ as well as $|\mathbf{E}|_{pp}$ are twice as large than in STL1.

IV. DISCUSSION

Excitation spread at smaller size scales may differ substantially from clinical observations at a coarser size scale. Clinically, mapping systems are not yet capable of resolving complex signals in the submillimeter range. The origin of CFAE has been demonstrated recently by a rule-based computer model derived from statistical distribution of connective tissue in microfibrosis [10]. Our work is based on singular histograms of atrial tissue obtained after electrophysiological experiments and it describes the behavior of local conduction parameters like FI, $\dot{\Phi}_{ep}$ and $|\mathbf{E}|_{pp}$ when the stimulus site is changed. Subregions A1 and A2 differ in microstructure: A1 represents electrically well coupled tissue in longitudinal and transverse directions with very few interstitial clefts. When applying the continuous cable theory, anisotropic and uniform conduction can be expected regardless the direction of the wavefront. The results described above confirm this; A2 is characterized by parallel oriented fiber bundles separated by substantial interstitial space. Wavefronts traveling through these neighboring fiber bundles will produce CFAE in the extracellular space when the individual activations are delayed with respect to each other. We have shown that the generation of such delays depends on the entering of the wavefront at the bifurcation site. Due to this mechanism, fractionated as well as uniform electrograms can be recorded at the same area depending on the pacing site. In addition it has been shown that signal and conduction parameters in A1 do not differ substantially in case of antegrade and retrograde conduction.

In A2, on the other hand, it was verified a much larger impact on all conduction parameters (Table II) as well as on the waveforms (Figure 4). This computer model is a very valuable tool to study directional effects on micropatterns of activation caused by recurrent interstitial cleft spaces. The results suggest that in experiments the analysis of $\dot{\Phi}_e$ and $\mathbf{E}$ combined with multisite pacing could give insights about type and size of fibrosis.

Acknowledgments

The authors would like to thank Michaela Janschitz and Verena Gruber for the help with the experimental work as well as Markus Absenger for providing the infrastructure and the support necessary to obtain the high resolution digital scans of the histological slices.

This work was supported by the grants P19993-N15 and SFB F3210-N18 from the Austrian Science Fund (FWF) as well as from the Extremadura regional funds of the project PRI06B186.

References

- [1]. Feinberg WM, Blackshear JL, Laupacis A, Kronmal R, Hart RG. Prevalence, age distribution, and gender of patients with atrial fibrillation. Analysis and implications. *Arch Intern Med.* 1995; 155(5):469–73. [PubMed: 7864703]
- [2]. Plank G, Hofer E. Model study of vector-loop morphology during electrical mapping of microscopic conduction in cardiac tissue. *Ann Biomed Eng.* 2000; 28(10):1244–52. [PubMed: 11144985]
- [3]. Hofer, E.; Sanchez-Quintana, D.; Plank, G.; Tischler, M. Normal and fractionated cardiac near fields and their relation to microstructure - an experimental approach. *Engineering in Medicine and Biology Society; Proceedings of the 25th Annual International Conference of the IEEE;* 2003; Sep. 2003 p. 51-54.
- [4]. Some Methods for classification and Analysis of Multivariate Observations. 1967; 1
- [5]. Lindblad DS, Murphey CR, Clark JW, Giles WR. A model of the action potential and underlying membrane currents in a rabbit atrial cell. *Am J Physiol.* 1996; 271(4):H1666–96. [PubMed: 8897964]
- [6]. Karlon WJ, Covell JW, McCulloch AD, Hunter JJ, Omens JH. Automated measurement of myofiber disarray in transgenic mice with ventricular expression of ras. *Anat Rec.* 1998; 252(4): 612–25. [PubMed: 9845212]
- [7]. Vigmond EJ, Hughes M, Plank G, Leon LJ. Computational tools for modeling electrical activity in cardiac tissue. *J Electrocardiol.* 2003; 36(Suppl):69–74. [PubMed: 14716595]
- [8]. Clark J, Plonsey R. A mathematical evaluation of the core conductor model. *Biophys J.* 1966; 6(1):95–112. [PubMed: 5903155]
- [9]. Hofer E, Keplinger F, Thurner T, Wiener T, Sanchez-Quintana D, Climent V, Plank G. A new floating sensor array to detect electric near fields of beating heart preparations. *Biosens Bioelectron.* 2006; 21(12):2232–9. [PubMed: 16384696]
- [10]. Jacquemet V, Henriquez CS. Genesis of complex fractionated atrial electrograms in zones of slow conduction: a computer model of microfibrosis. *Heart Rhythm.* 2009; 6(6):803–10. [PubMed: 19467508]

Fig. 1.

(a) Image of ROI of a Rabbit heart. (b) Micrograph of the selected area. Cyan (trichrome staining) corresponds to connective tissue. Subregions A1 and A2 relate to two types of configuration of interstitial space: A1 - densely packed and electrically well coupled tissue and, A2 - fibers uncoupled by connective tissue and interstitial space oriented parallel.

Fig. 2. Depolarization wavefronts traveling through subregions A1 (left column) and A2 (right column) from three different stimulus sites (see Figure 1b). Color coding refers to the transmembrane potentials (black and red mean resting state depolarized states, respectively). The images are rotated in 90° in relation to Figure 1b.

Fig. 3.

Vector-loops of $\mathbf{E}$ and $\dot{\Phi}_e$ in subregion A1 originated by stimulus STL1 (a) and STL3 (b). Color coding refers to the degree of fractionation $FI = 1$ (black) and 2 (blue).

Fig. 4.

Vector-loops of $\mathbf{E}$ and $\dot{\Phi}_e$ in subregion A2 originated by stimulus STL1 (a) and STL2 (b). Color coding refers to the degree of fractionation FI = 1 (black), 2 (blue) and 3+ (red).

TABLE I

Changes of conduction parameters in subregion A1. Fractionation index FI = 1 (N1), 2 (N2) and more than 3 (N3+), mean μ and standard deviation σ of $\dot{\Phi}_{epp}$ and $|E|_{pp}$.

Stimulus site	FI			$\dot{\Phi}_{epp}$ (N = 56)		$ E _{pp}$ (N = 56)	
	N1	N2	N3+	μ	σ	μ	σ
STL1	56	0	0	70.45	14.08	84.36	9.610
STL2	52	1	3	64.98	11.13	82.92	10.03
STL3	53	3	0	54.14	12.71	71.95	9.370

TABLE II

Changes of conduction parameters in subregion A2. Fractionation index FI = 1 (N1), 2 (N2) and more than 3 (N3+), mean μ and standard deviation σ of $\dot{\Phi}_{epp}$ and $|E|_{pp}$.

Stimulus site	FI			$\dot{\Phi}_{epp}$ (N = 76)		$ E _{pp}$ (N = 76)	
	N1	N2	N3+	μ	σ	μ	σ
STL1	22	33	21	24.59	10.81	34.73	10.89
STL2	57	17	2	60.05	20.63	66.98	18.77
STL3	25	37	14	47.51	15.42	47.48	12.42