Impact of Mechanically-Induced Fibrosis on Atrial Electromechanical Function

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Abstract

Atrial fibrillation is accompanied by remodelling processes on the structural, electrophysiological, and contractile levels. These alterations result at least in part from mechanical overload. In order to investigate the effects of mechanical forces on atrial cell and tissue mechano-electric functions, we established two human in-vitro models, one at cell and the other at tissue level.

The cellular model consists of functional atrial- and ventricular-like cardiomyocyte populations from human induced pluripotent stem cells, to characterize their electrical and mechanical functions in various mechanical environments. The tissue-based model consists of slices that are electrically and mechanically stimulated in biomimetic chambers for up to 7 days. We hypothesize that mechanical overload induces fibrosis, mimicking the condition of atrial tissue from patients having sustained atrial fibrillation. We aim to use this model for an in-depth characterization of the effects of mechanical.

By compiling all data into a computational model, we propose to establish a digital slice model, in which parameters such as collagen distribution, tissue and extracellular matrix stiffness, as well as excitation can be changed independently to assess their contribution to passive and active mechanics and their effects on electrophysiological properties of atrial tissue slices over time.

1. Introduction

The supraventricular tachyarrhythmia atrial fibrillation (AF) is characterized by uncoordinated, high-frequency electrical activation of the atria, that are conducted to the ventricular chambers in an irregular manner. The most common triggers of AF are ectopic pulses originating in the myocardial sleeves within the wall of the pulmonary veins shortly before they end in the left atrium. When encountering a proarrhythmic substrate, for example a fibrotic region, such a trigger can perpetuate abnormal impulse conduction by reentry. Episodes of atrial fibrillation may initially terminate spontaneously after a few seconds up to several days. The high irregular activation frequency of the atria is associated with profound electrophysiological, structural and mechanical remodeling processes, including action potential shortening, development of fibrosis and atrial dilatation. All of these alterations promote reentry and thereby enhance the incidence of AF recurrence potentially leading to permanent AF.

2. Role of mechanical forces in atrial fibrillation

While the heart performs its blood pumping function, all cells within the myocardium are exposed to changing mechanical forces. Under pathophysiological conditions such as in heart failure or valvular diseases, the atria may become dilated by chronic stretch. Stretch is detected by mechanosensors as for example stretch-activated channels, and is transduced into specific responses. Acute stretch may be proarrhythmic as demonstrated in isolated, Langendorff-perfused hearts of rabbit. Increased pressure in a balloon inserted into the left atrium of the heart was used to induce acute atrial dilation, which facilitated induction of AF [1]. Moreover, this pressure increase was closely followed by a shortening in atrial refractory period and a similar reduction in atrial monophasic action potential duration [1]. AF inducibility upon stretch was inhibited by gadolinium (Gd^{3+}), a non-selective blocker of stretch-activated channels, in a concentration-dependent manner, suggesting the involvement of stretch-activated channels in AF facilitation [2]. Many stretch-activated channels are cation non-selective. They may be proarrhythmic by enhancing Ca^{2+} entry and delayed afterdepolarization as demonstrated in rat atria [3]. Interestingly, neither Gd^{3+} nor the more selective blocker of cation non-selective stretch-activated channels GsMtx-4, a peptide toxin from the tarantula *Grammostola spatulata*, affected the reduction in atrial refractory period associated with increased intra-atrial pressure [2,4]. Since refractory period and action potential duration are closely associated, it may be concluded that AF-induced action potential shortening during acute stretch is probably not directly related with stretch-activated channels.

The effects of chronic stretch on the heart have been studied extensively in *in vivo* models of left-ventricular

overload. In the trans-aortic constriction model, the diameter of the aorta is reduced by banding of the transverse aorta. This leads to profound ventricular hypertrophy and fibrosis [5]. Although atrial pressure overload and atrial fibrillation are clinically correlated (for recent review see Li *et al.* [6]), few experimental models directly address effects of mechanical stimulation on atrial cells and tissue, in particular mechanically-induced fibrosis in atrial tissue.

Within the PersonalizeAF consortium, we have developed two different experimental tools to study the pro-arrhythmic effects of acute and chronic mechanical overload. The first approach aimed at differentiating atrial cardiomyocytes from human induced pluripotent stem cells (hiPSC). These cells - either alone or in co-culture with fibroblasts or immune cells will represent a powerful *in vitro* model to investigate the effects of mechanical alterations encountered in mechanically overloaded tissues: *e.g*. stretch (use of stretchable culture substrates) and matrix stiffening (use of gels with various stiffnesses). In the second approach, we utilize organotypic tissue slices to investigate the effects of mechanically-induced fibrosis on atrial electromechanical function.

3. Model of hiPSC-derived cardiomyocytes

Since Takahashi and Yamanaka's discovery of hiPSC [7], many protocols to differentiate them into cardiomyocytes have been established [8]. However, the generation of a homogeneous and functional population of hiPSC-derived atrial cardiomyocytes is still limited. In this work, we differentiated hiPSC atrial- and ventricular-like cardiomyocytes in parallel. For differentiation into an atrial phenotype retinoic acid (1 µM) was added to the cell culture from day 3 to day 8. The two cardiomyocyte populations were compared on day 30.

Both cell types beat spontaneously, albeit at different frequencies, *i.e.* at 1.4 ± 0.3 and 0.4 ± 0.1 Hz for atrial- and ventricular-like cells, respectively. Representative traces of action potentials and contractions are shown in Fig. 1. Resting membrane potentials (-57.4 ± 6.1 mV and -58.7 ± 7.3 mV, atrial *vs.* ventricular cells) and action potential amplitudes (83.9 ± 18.9 mV and 81.4 ± 19.4 mV, atrial *vs.* ventricular cells) were not different between the two cardiomyocyte phenotypes (all values are means ± standard deviation). Action potential duration at 90 % of repolarization was lower in atrial-like compared to ventricular-like cardiomyocytes (220.6 ± 27.3 ms *vs.* 499.7 ± 40.5 ms, n = 4 each, $p<0.0286$). In addition, the kinetics of contractions were different between these cells as evidenced by shorter time-to-peak intervals and shorter time from half-maximum contraction to half-maximum relaxation in atrial- *vs.* ventricular-like cells. It must be emphasized in this context, that both electrical and mechanical kinetics are known to be frequency-dependent, but the expected extent of change due to the different beating rates of the two cell types is much smaller than the differences observed here. We therefore conclude that the differences in electrical and mechanical kinetics allow clear distinction between the two phenotypes of hiPSC-derived cardiomyocytes.

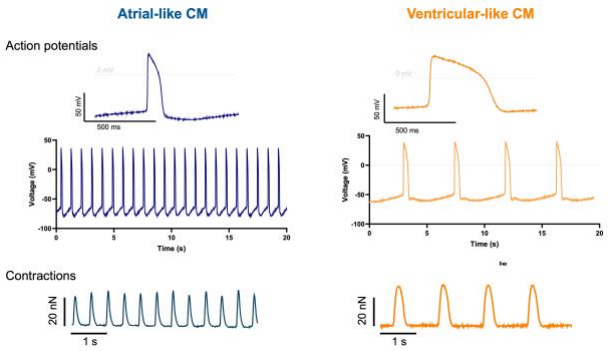

Figure 1: Spontaneous electrical and mechanical activity of hiPSC-derived cardiomyocytes. Left: atrial-like cells, right: ventricular-like cells. Upper traces: action potentials recorded with sharp microelectrodes at 37°C, lower traces: contractions recorded with the nano-indentation technique at 37°C. Please note the difference in time scale between the recordings of action potentials and contractions.

4. Model of atrial tissue slices for studying mechanically induced fibrosis

In order to investigate the development of fibrosis due to chronic mechanical overload, we have developed the model of atrial tissue slices (see also [9]). In brief, a small chunk of atrial tissue (edge length 10 mm) was embedded in an agar block and cut into 400 µm thick slices with a vibratome. These were mounted in a custom-made culture chamber [10], in which they were pre-stretched and electrically stimulated (Fig. 2A). Simultaneously, we recorded active and passive force development. In the presence of 20 nM cortisol, the slices survived up to 5-7 days in culture. The preparations contracted upon electrical stimulation and exhibited physiological responses such as the Frank-Starling phenomenon, *i.e.* increased contraction amplitudes with increased preload. Exposure to isoprenaline (300 nM) resulted in a robust positive inotropic effect. These results indicated, that the slices were viable and suitable for studying the effects of chronic preload.

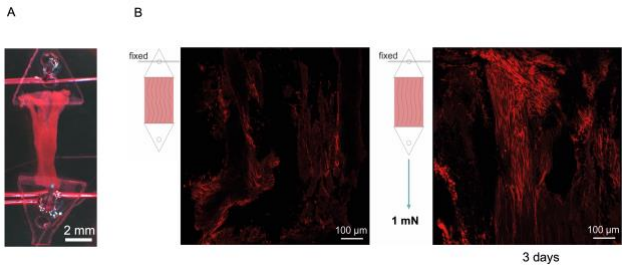

Figure 2: Living culture of atrial tissue slices in biomimetic chambers as a model to study mechanically-induced fibrosis. A, tissue slice (human atrium) glued between two triangles and mounted in the culture chamber. B, histological staining of collagen (using a genetically-encoded probe based on the collagen binding protein CNA35 fused to tdTomato) in a human atrial tissue slice maintained in the biomimetics chamber for 4 days. Left: control conditions (basal preload 1 mN); right: effect of 1 mN extra mechanical load. Note increased production of collagen in response to additional preload within 3 days of culture.

These initial experiments were carried out in slices that were kept at a basal preload of 1 mN so that we were able to measure contractile responses to electrical stimulation. In some preparations, we then increased the preload by 1 mN at day 1. After 4 days of culture, we stained the slices for collagen (see Fig. 2B). Slices cultured under increased preload condition, were characterized by larger collagen-positive area and higher intensity of the collagen staining compared to control slices. From these preliminary results we conclude that atrial tissue slices are a promising model to study mechanically-induced fibrosis.

5. Outlook

Because of ample evidence in the literature that mechanical forces in form of acute and chronic stretch may contribute to initiation and maintenance of atrial fibrillation, we have developed and characterized two human cell-based models in which to study these phenomena more closely. These experimental tools will allow us to investigate the pro-arrhythmic effects of acute and chronic mechanical overload. We also aim to recapitulate structural and mechanical observations in an *in-silico* model for an in-depth investigation of the interplay between mechanical forces (both active and passive) and tissue remodeling.

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