INNOVATIVE TECHNIQUES AND NEW INSIGHTS

Studying cardiac ionic currents and action potentials in physiologically relevant conditions

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Abstract

Cardiac arrhythmias are associated with various forms of heart diseases. Ventricular arrhythmias present a significant risk for sudden cardiac death. Atrial fibrillations predispose to blood clots leading to stroke and heart attack. Scientists have been developing patch-clamp technology to study ion channels and action potentials (APs) underlying cardiac excitation and arrhythmias. Beyond the traditional patch-clamp techniques, innovative new techniques were developed for studying complex arrhythmia mechanisms. Here we review the recent development of methods including AP-Clamp, Dynamic Clamp, AP-Clamp Sequential Dissection, and Patch-Clamp-in-Gel. These methods provide powerful tools for researchers to decipher how the dynamic systems in excitation-Ca^{2+} signaling-contraction feedforward and feedback to control cardiac function and how their dysregulations lead to heart diseases.

Keywords: action potential, arrhythmia, cardiac myocyte, patch-clamp, electrophysiology.

Resumen

Las arritmias cardiacas están asociadas a diferentes tipos de enfermedad cardiaca. Las arritmias ventriculares constituyen un alto riesgo de muerte súbita. La fibrilación auricular predispone a coágulos sanguíneos que pueden producir accidentes cerebrovasculares e infarto miocárdico. Los científicos han desarrollado la técnica de patch-clamp para estudiar los canales iónicos y los potenciales de acción (PAs), que constituyen la base de la excitación y las arritmias cardiacas. Además de las clásicas técnicas de patch-clamp, se desarrollaron técnicas innovativas para estudiar los mecanismos complejos de las arritmias. En este trabajo, describimos diferentes métodos recientemente desarrollados tales como AP-clamp ("clampeo" del PA), Dynamic Clamp ("clampeo" dinámico), AP-Clamp Sequential Dissection, (disección secuencial del "clampeo" del AP), y Patch-Clamp-in-Gel (Patch clamp en gel). Estos métodos constituyen herramientas poderosas para descifrar cómo los sistemas dinámicos que constituyen la excitación-las señales de Ca^{2+} y la contracción, se retroalimentan para controlar la función cardiaca y cómo sus alteraciones llevan a la enfermedad cardiaca.

Palabras clave: potencial de acción, arritmias, miocitos cardiacos, patch -clamp, electrofisiología.

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Introduction

Cardiac action potentials drive the rhythmic heartbeats and dysregulation of action potentials can lead to cardiac arrhythmias. In cardiomyocytes, the action potential (AP) is determined by myriad ionic currents that work in concert to control the rise and fall of the transmembrane voltage. The AP timing and voltage profile is dynamically shaped by the counterbalancing effects of inward currents versus outward currents. Researchers have developed various patch-clamp techniques to investigate ion channels and action potentials to understand their roles in cell function and diseases. Traditionally, the voltage-clamp technique is used to determine ion channel properties and the current-clamp technique is used to record the cell's action potential. In this minireview, we will focus on the recent development of innovative techniques for studying the dynamic ionic currents under the AP in physiologically relevant conditions that closely mimic the in-situ environment for studying how the dynamic ionic currents contribute to shaping the action potential and arrhythmogenic activities.

AP-Clamp techniques to study the dynamics of ionic currents during cardiac AP cycle

To study the dynamics of ionic currents during the AP cycle, AP-clamp techniques were developed by using the AP wave form to voltage-clamp the cell. [5, 6, 7, 8, 9] In comparison, traditional voltage-clamp typically uses a rectangular voltage pulse to record the current flowing through a particular ion channel and study its electrophysiological properties (i.e., ion selectivity, conductance, gating, activation, inactivation, recovery, etc.) at constant voltage. These properties were then put into a mathematical model using the approach pioneered by Hodgkin–Huxley [10], which is then used to calculate the profile of the ionic currents during an AP. The AP-clamp technique directly measures the ionic current during the AP using the following experimental protocol (Figure-1A): (1) Under current-clamp (I = 0) mode, pace the cell to reach steady-state and record the cell's steady-state AP. (2) Switch to voltage-clamp mode, and use this AP waveform as the voltage command to voltage-clamp the cell and repeat the cycle until reaching steady-state; the net current output – the sum of all currents - should be zero at steady-state, which serves as the reference current (IRef). (3) Block an ionic current by using its specific blocker to remove it from the net current output; this missing current is seen in the compensation current (IComp) injected by the amplifier to maintain the AP waveform. (4) The difference between the reference and the compensation current (IDiff = IRef –IComp) shows the ionic current that was originally flowing during the AP prior to the blocker application. The above protocol is used with some variations: canonical AP-Clamp using a pre-recorded AP waveform to clamp different cells [11], reconstructed AP-Clamp using a reconstructed AP waveform to study the ion channel kinetic properties [12], or self AP-Clamp using the AP recorded from the same cell to do AP-clamp to mimic the physiological condition in the cell. [3, 13].

There are fundamental differences between the AP-clamp techniques and the traditional voltage-clamp techniques. The AP-clamp records an ionic current over a changing voltage during an AP, whereas the traditional voltage-clamp records the current at constant voltage. AP-clamp experiments record the 'absence of the specific current' by blocking one single current using its specific blocker while allowing all other currents to flow during AP in physiological condition. In contrast, traditional voltage-clamp experiments record a single current using non-physiological condition that suppress all other currents (i.e., using rectangular pulse voltage protocols, simplifying solutions to select ion species, blocking other contaminating currents using pharmacological inhibitors, etc.) Furthermore, the intracellular Ca^{2+} transient during AP-clamp can be preserved by eliminating exogenous Ca^{2+} buffer in the pipette solution to mimic the physiological condition in cardiomyocytes.

AP-Clamp allows direct recording of the ionic currents during the dynamically changing action potential. For example, Horvath et al. [14] recorded the TTX-sensitive Na^+ current under AP-Clamp, which shows a distinct profile that clearly separates the late Na^+ current (I_{NaL}) from the fast Na^+ current. The I_{NaL} profile under AP-Clamp drastically differs from the I_{NaL} recorded under rectangular pulse voltage-clamp where the I_{NaL} merges with the fast Na+ current and the two are hard to separate [15]. The AP-Clamp data reveals that the I_{NaL} during cardiac AP is much larger than previously estimated. Using AP-Clamp, Banyazs et al. [16] discovered that the L-type Ca^{2+} channel ($I_{Ca\text{-}L}$) has

rapid deactivation and reactivation during the AP notch; this causes transmural difference in $I_{Ca\text{-}L}$ because the epicardium has a more pronounced AP notch than the endocardium. Combined with Ca^{2+} measurement, AP-clamp with Ca^{2+} cycling provides a powerful method for studying the dynamic interplay of Ca^{2+}, ionic currents, action potentials and arrhythmogenic activities. [11, 13, 17]. For example, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is an important regulator of ion channels including $I_{Ca\text{-}L}$ and I_{NaL}. AP-clamp with Ca^{2+} cycling experiments revealed the dynamic profile of I_{NaL} during cardiac AP and its prominent role in arrhythmogenesis. [14, 18] The AP-clamp with Ca^{2+} cycling method also enables studying how intracellular Ca^{2+} transient modulates the Ca^{2+}-sensitive currents, such as the L-type Ca^{2+} current and the Na / Ca^{2+} exchanger current, during the cardiac AP cycle. [13]

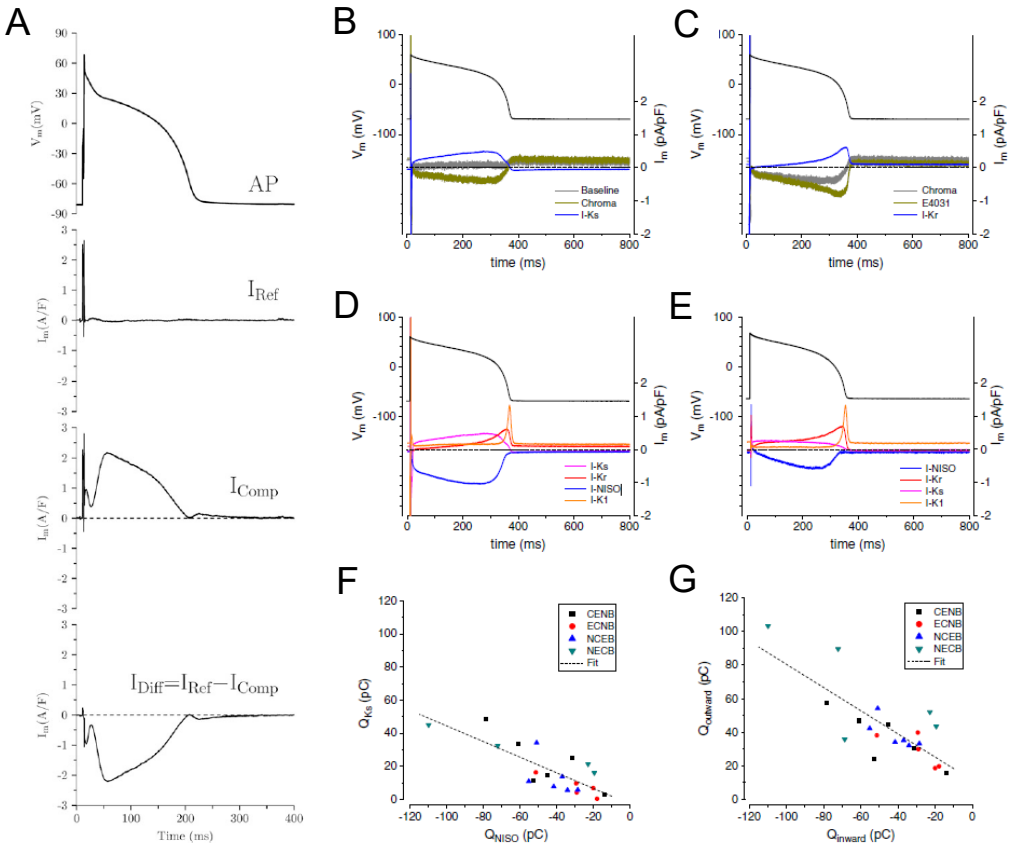

Figure 1. AP-Clamp method for studying the dynamics of ionic currents during cardiac AP.
(Figures reconstructed from Banyasz et al., 2011 [3].)

A. Schematic of AP-clamp technique: (1) Record the cell's steady state AP. (2) Use this AP as the voltage clamp command to record the total net current output, I_{Ref}. (3) Isolate a current by using its specific blocker to remove it from the total net current output, and record the compensation current from the amplifier, I_{Comp}. (4) The current of interest is obtained as the difference current: $I_{Diff} = I_{Ref} - I_{Comp}$.
B. The first current I_{Ks} is obtained by subtracting the compensation current recorded with chromanol-293B from the baseline current.
C. The second current I_{Kr} is obtained by subtracting the compensation current recorded with E4031+chromanol from the first compensation current recorded with chromanol.
D. AP-Clamp Sequential Dissection recording of 4 currents, I_{Ks}, I_{Kr}, I_{K1} and $I_{Ca\text{-}L}$ using the corresponding channel blockers chromanol-293B, E4031, Ba^{2+} and nisoldipine.
E. AP-Clamp Sequential Dissection recording of 4 currents, I_{Ks}, I_{Kr}, I_{K1} and $I_{Ca\text{-}L}$ from another cell, showing smaller current densities in all currents.
F. Correlation between the charges carried by inward $I_{Ca\text{-}L}$ and outward I_{Ks}.
G. Correlation between the charges carried by inward $I_{Ca\text{-}L}$ and the sum of charges carried by the outward I_{Ks}, I_{Kr}, I_{K1} currents.

Dynamic Clamp for studying dynamic interaction of the AP and the underlying currents

The Dynamic Clamp method was developed to investigate the interplay between ionic currents and action potentials in cardiomyocytes. The Dynamic Clamp electrically connects two cells (cell-A, cell-B) to allow intercellular electrical coupling. The ionic current in cell-B can be manipulated genetically or pharmacologically, or custom designed using computational model simulation. This current from cell-B is injected into cell-A to study its effect on modifying the cell's action potential. In the recent decades, Dynamic Clamp has evolved to include diverse techniques such as coupling clamp, model clamp, and dynamic AP-Clamp. [19] The technical implementations of Dynamic Clamp require sophisticated hardware and software, which had been nicely summarized in previous reviews. [19, 20] Here we briefly mention the advantages as well as limitations of the method.

Dynamic Clamp provides powerful tools to manipulate the injected current to study its effect on modifying the action potential in real-time. The injected current can be recorded from another cell expressing a native or mutant ion channel or from a mathematical model simulation. For example, Berecki et al. [21] used coupling clamp to study how various mutations in the rapid rectifier K^+ current (I_{Kr}) affect the AP. They removed native I_{Kr} using specific pharmacological blocker E-4031 in a cardiomyocyte (cell-A). They expressed mutant I_{Kr} in a HEK-293 cell (cell-B), recorded the mutant I_{Kr} current under AP-clamp (using the AP from cell-A), and injected it into cell-A. This intercellular coupling incorporates the mutant I_{Kr} into the AP in real time. They found that wild-type I_{Kr} injection restored the original APD, but R56Q mutation prolonged APD leading to long-QT2 syndrome. Using this approach, they also found that cardiac Na^+ channel (SCN5A) mutations Y1795C and A1330P prolonged APD leading to long-QT3 syndrome. [22] Using the model clamp approach, Altomare et al. [23] blocked the native I_{Kr} in a cardiomyocyte (cell-A); generated an artificial current using a computational model of I_{Kr} (cell-B); and then injected the artificial current to study its effect on the AP profile. They found that the model generated I_{Kr} current could adequately replace the native I_{Kr} to recover the original APD, attesting to the accuracy of the I_{Kr} model. Then they manipulated the model parameters to study the effect of each parameter on modifying the I_{Kr} and the AP profile. They found that the APD variability is sensitive to the steady-state inactivation more than to the activation. This approach enables systematic and detailed studies on how the voltage-time dependent channel properties influence the current and the AP. Hence, Dynamic Clamp providing a powerful tool to simulate the effects of mutations or drugs on modifying the channel properties and the consequences on the AP and arrhythmogenic activities.

Dynamic Clamp also allows investigating the mechanistic details in the interplay between AP profile and ionic currents. Experiments injecting I_{to} to manipulate AP notch revealed that the 'spike-and-dome' and the plateau height critically determine the AP duration, which demystifies why different animal species with different AP profiles have differential response to β-adrenergic stimulation. [24] Dynamic Clamp experiments also showed that the β-adrenergic upregulation of the funny current (I_f) in cardiac pacemaker cells accounts for 60% of the AP interval decrease and 41% of the AP firing rate increase for the fight-or-flight response. [25] Recently, Dynamic Clamp is used to inject an artificial I_{K1} current into human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs, with immature AP profile) to simulate "adult-like" AP response to drugs. [26]

A major limitation of the Dynamic Clamp method is due to that the injected current uses electron as the charge carrier, which differs from the ion species (i.e., Na^+, K^+, Ca^{2+}, etc.) that flow through selective ion channels. The ionic currents are necessary for generating the ionic gradients that set the electrochemical potentials across the cell membrane. Restoring the ionic homeostasis is needed for maintaining the cell function during cardiac cycle. However, the roles of ionic currents in establishing ionic homeostasis cannot be mimicked by the electron injection used in Dynamic Clamp.

AP-Clamp Sequential Dissection to study the counterbalance of ionic currents during AP

AP-clamp Sequential Dissection technique, nicknamed 'Onion-Peeling', was further developed from AP-Clamp to record many different ionic currents in the same cell. [3, 1] Previous patch-clamp techniques record only one current in any one cell. The AP-clamp Sequential Dissection uses a series

of channel blockers to sequentially dissect out many ionic currents one by one in a single cell under self AP-Clamp. The experimental protocol mainly consists of the following steps. First, the cell is placed under AP-clamp; a specific blocker is applied to dissect out its corresponding ionic current (Figure-1B). Next, a second blocker is applied to dissect out the second current (Figure-1C). Next, a third blocker is applied to dissect out the third current, etc. Figure-1D shows 4 ionic currents (I_{Ks}, I_{Kr}, I_{K1}, $I_{Ca\text{-}L}$) dissected out by sequentially applying the specific blockers for each current. [3] A technical limitation for the AP-Clamp Sequential Dissection method is the specificity of channel blockers. To minimize the off targets effects, one can use low dosage of blockers that is usually more specific, and then scale to the full current density. To preserve the Ca^{2+} transient during 'onion-peeling', the Ca^{2+} channel blocker is used only at the end after other channel blockers.

The unprecedented ability to measure many different currents in the same cell enables studying how the inward currents versus the outward currents counterbalance to shape the cell's AP. Studies using this technique show that the contribution of different ionic currents to shaping the AP may dramatically change under various conditions such as beta-adrenergic stimulation or heart disease development. [27, 1] AP-clamp Sequential Dissection can be used to study many different ionic currents and integrate them at the individual cell level, which provides a comprehensive view on the individual cell electrophysiology as well cell-to-cell variability at the tissue level.

Patch-Clamp-in-Gel to study the mechanical load regulation of AP and ionic currents

The heart pumps blood against the mechanical load from arterial resistance, and increased load may alter cardiac electrophysiology and contribute to arrhythmias. To study mechanical load effects on cardiomyocytes, the Cell-in-Gel technology was developed to embed isolated cardiomyocytes in a 3-D hydrogel made of viscoelastic polymer matrix. [28] Mechanical analyses show that when the cell is contracting in-gel, the viscoelastic gel exerts 3-D mechanical stress on the cell (Figure-3A, longitudinal tension, transverse compression, and surface traction with normal and shear stress). [2, 29, 30, 31] To study the mechanical load effects on electrophysiology, the Patch-Clamp-in-Gel technique was further developed to control the mechanical load on the cell in patch-clamp experiments. [4] Figure-3B shows a schematic of the Patch-Clamp-in-Gel technique. First, perform standard patch-clamp experiment (whole-cell configuration) in a cell bathed in Tyrode's solution containing polyvinyl alcohol (PVA). Pace the cell to reach steady-state and record the APs (Figure-3C, load-free control). Next, add tetraboronate-polyethylene glycol (4B-PEG) to crosslink PVA to form polymer matrix that embeds the cell-electrode assembly in a viscoelastic hydrogel (Figure-3D, cell in-gel). As the hydrogel forms gradually over minutes, the AP duration (APD_{90}) initially became longer in the cell contracting under moderate load in softer gel (Figure-3E, 3G), followed by development of APD alternans in the cell contracting under higher load in stiffer gel (Figure-3F, 3G). These data demonstrate that the mechanical load on cardiomyocytes during cell contraction profoundly regulates the cell's electrophysiological properties.

Patch-Clamp-in-Gel experiments show that the cardiomyocyte under mechanical load has increased L-type Ca^{2+} current, increased transient outward K+ current, decreased inward rectifier K^+ current, and prolonged APD. [4] These changes allow more Ca^{2+} to enter the cell during the AP cycle, causing elevated intracellular Ca^{2+} content that increases the SR Ca^{2+} release and cytosolic Ca^{2+} transient. Increased Ca^{2+} transient gives rise to enhanced contractility of the cardiomyocyte contracting under mechanical load. 31 32 Taken together, these experimental data reveal the mechano-chemo-electro-transduction (MCET) mechanisms that enable cardiomyocytes to upregulate contractility to compensate for the increased load. Furthermore, systematic studies applying different levels of mechanical load on cardiomyocytes show that MCET provides feedback loops in the dynamic system of excitation- Ca^{2+} signaling-contraction (E-C) coupling, which enables autoregulation of E-C coupling and contractility in cardiomyocytes. [31, 32] Studies also show that exceedingly high load causes dysregulation of MCET, spontaneous Ca^{2+} activities, and discordant alternans in APD and Ca^{2+} transient, which provide substrate to cardiac arrhythmias. [4, 28, 32]

New insights on the complex changes of cellular electrophysiology in health and diseases

Cardiac action potential is shaped by myriad ionic currents flowing in or out of the cell in a time-dependent manner. The AP-clamp Sequential Dissection method enables recording many different currents from a single cell, which is a significant step beyond the traditional way of recording only one current in any one cell. The ability to measure different currents in a single cell revealed hitherto unknown characteristics of the ionic currents in individual cardiomyocytes: coordination of the currents within a cell and large variations between different cells. In the first Onion-Peeling study [3], four different ionic currents (I_{Ks}, I_{Kr}, I_{K1}, and $I_{Ca\text{-}L}$) were measured from [21] ventricular myocytes isolated from healthy guinea pig heart. The data reveal that in a single cell, the inward currents and the outward currents are coordinated to produce a normal AP profile. However, different cells have large variations in the amplitudes of currents (Figure-1D, 1C). Interestingly, despite the large cell-to-cell variation (Figure-1F, 1G), the changes in the inward and the outward currents in each cell are coordinated to keep the normal AP profile across the individual cells in the healthy heart.

In failing hearts, the coordination between ionic currents may breakdown. Study of the ischemic heart failure (HF) [1] shows that the ionic currents in cardiomyocytes from the infarct border and remote zones undergo complex remodeling in HF (Figure-2A). The ventricular myocytes isolated from different zones show different action potentials (Figure-2B). AP-Clamp Sequential Dissection was used to record eight different ionic currents during the cell's AP under physiologically relevant condition (Figure-2C). Compared with healthy control, infarct-remote zone myocytes showed increases in the late Na^+ current, the Ca^{2+}-activated K^+ current, the Ca^{2+}-activated Cl^- current, but a decrease of the rapid delayed rectifier K^+ current and an altered Na^+/ Ca^{2+} exchange current profile (Figure-2D, 2E). In the infarct-border zone myocytes, additional changes occurred with a decrease of the L-type Ca^{2+} current and a decrease of the inward rectifier K^+current (Figure-2D, 2F). The changes in any individual current are relatively small, but the integrated impacts shift the balance between the inward currents and the outward currents to shorten AP in the border zone but prolong AP in the remote zone. Such differential remodeling increases the heterogeneity and dispersion of action potential durations, providing substrates to cardiac arrhythmias. Another study using AP-clamp Sequential Dissection shows that I_{Kr} and I_{NaL} are counterbalancing currents during the AP and they covary in a concerted manner in individual ventricular myocytes. [33] Moreover, the ratio of I_{Kr}/I_{NaL} is altered in HF, long QT and short QT syndromes, and restoring the I_{Kr}/I_{NaL} balance may represent a therapeutic strategy. Hence, the AP-clamp Sequential Dissection method provides a unique and powerful tool for studying the complex changes of cardiac electrophysiology in heart diseases as well as the complex effects of drug therapies.

In conclusion, the innovative techniques reviewed here provide powerful tools for investigating the complex mechanisms of cardiac arrhythmias. The AP-clamp techniques allow direct recording of the dynamic ionic currents during the action potential in physiologically relevant conditions. Dynamic Clamp provides versatile tools to manipulate ionic currents to study how they affect the AP profile. AP-Clamp Sequential Dissection enables recording of many different ionic currents in the same cell to study how they integrate to shape the cell's AP. Patch-Clamp-in-Gel provides a new tool for studying how the mechanical load during cell contraction feedback to autoregulate the E-C coupling systems. Studying E-C coupling as an autoregulatory feedback system will help deciphering how the heart autoregulates contractility in response to physiological load changes, and how pathological overload such as hypertension leads to cardiac arrhythmias and heart failure.

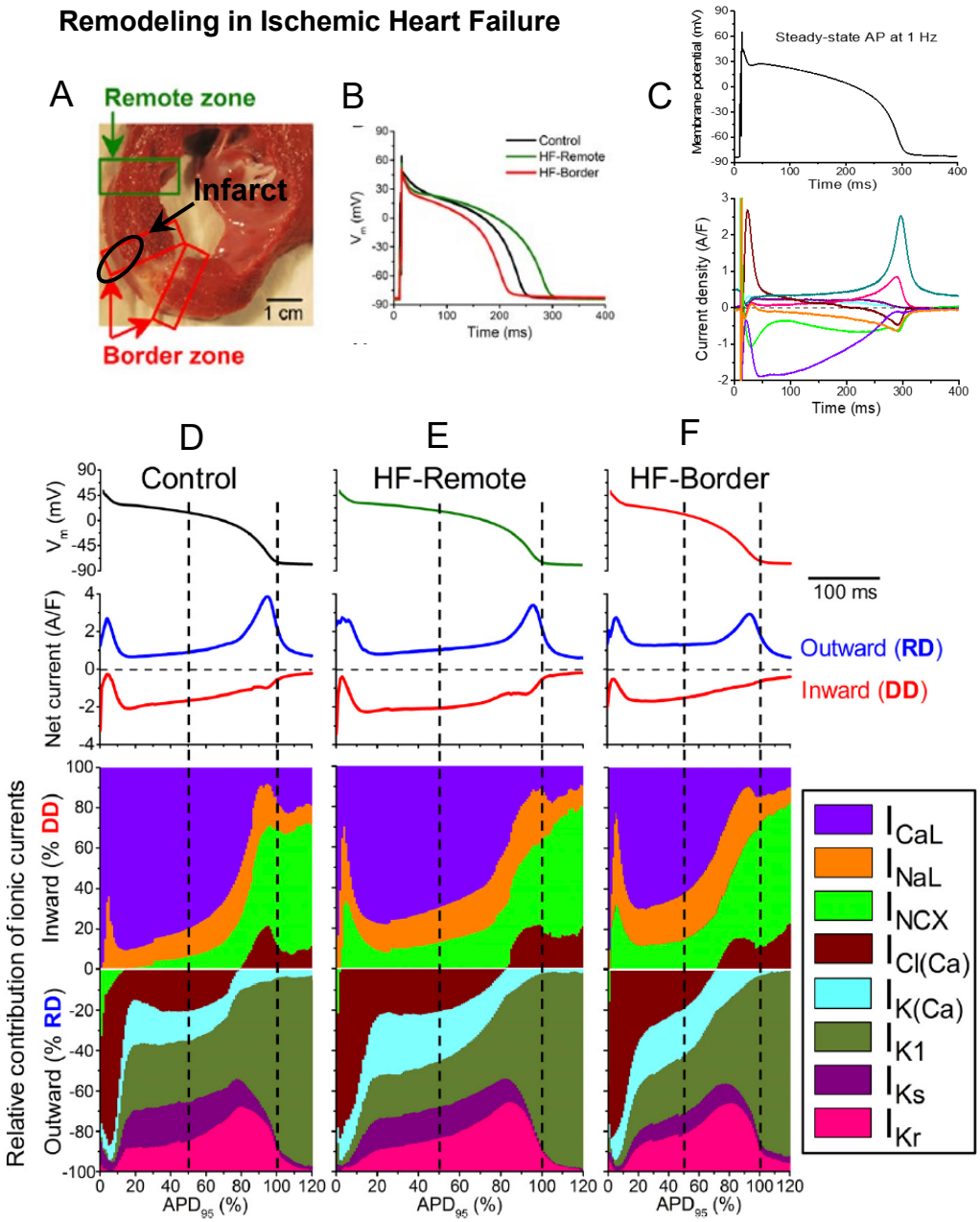

Figure 2. AP-Clamp Sequential Dissection to study complex changes in electrophysiology.
(Figures reconstructed from Hegyi et al., 2018 PNAS [1].)
(A) Ischemic heart failure (HF) showing the infarct, border zone, and remote zone.
(B) Representative APs from control, HF-remote, and HF-border cardiomyocytes at 36°C.
(C) AP-Clamp Sequential Dissection recording of 8 ionic currents from the same cell.
Dynamic ionic currents during the AP were recorded from the control (D), the HFremote zone, and the HF-border zone (F). The contribution of each inward and outward current was normalized to the momentary total outward (RD) or inward (DD) membrane current calculated based on the measured eight ionic current densities. Ionic currents measured in each cell were also normalized to the corresponding APD95. Bottom panels show the fingerprinting of ionic currents using AP-Clamp Sequential Dissection.

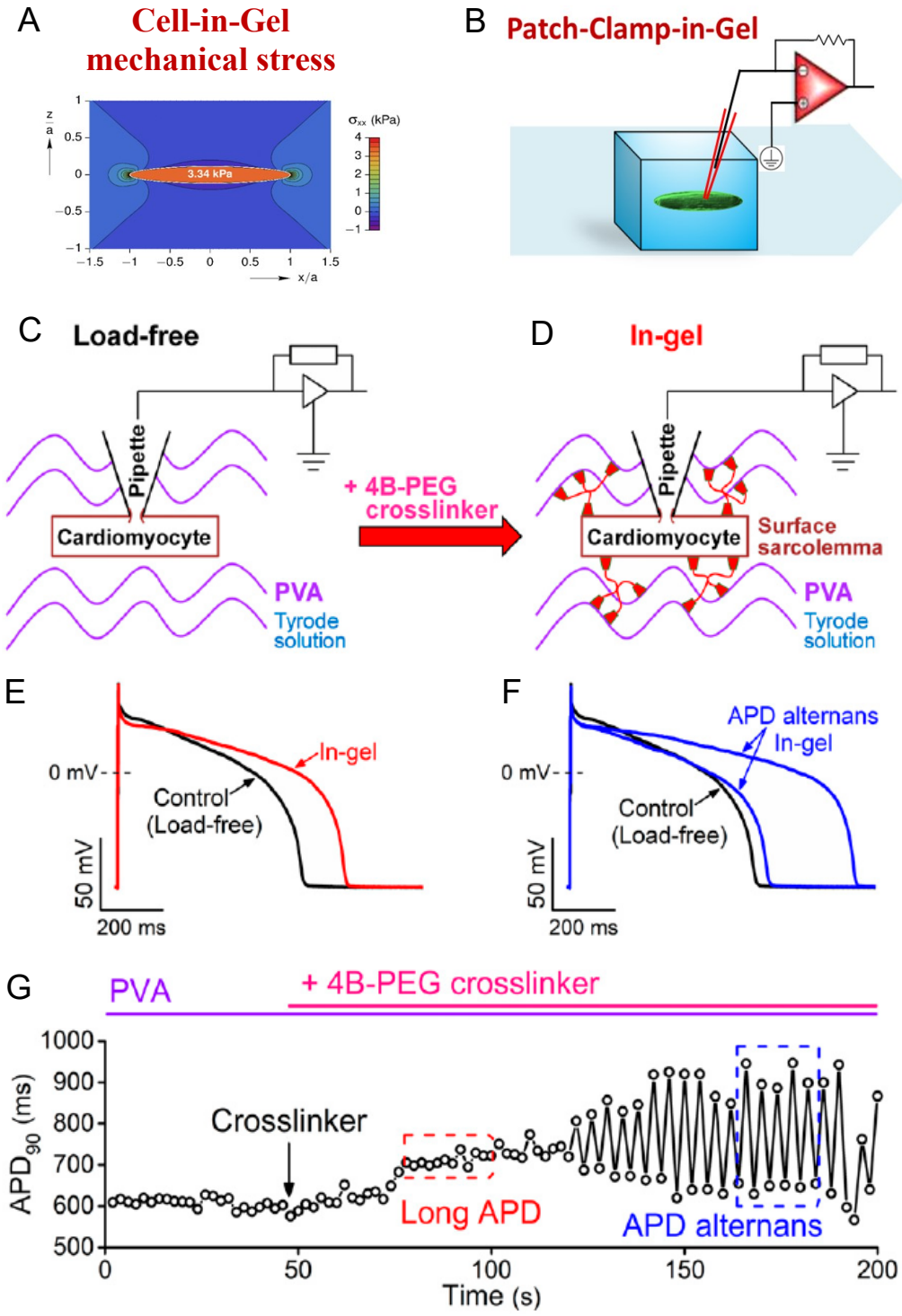

Figure 3. Patch-Clamp-in-Gel to study mechanical load effect on regulating ion channels and AP.
(Figures reconstructed from Kazemi-Lari et al., 2021[2]; and Hegyi et al., 2021[4].)
(A) Schematic of the Patch-Clamp-in-Gel method. (B) Cell-in-Gel system applies 3-D mechanical stress on the cardiomyocyte during contraction. (C) Patch-clamp experiments were performed in a cardiomyocyte in Tyrode solution containing PVA (load-free control). (D) Then, 4B-PEG cross-linker was added to form a 3-D hydrogel around the cell to apply mechanical load during cell contraction. (E) APD was prolonged in the cell contracting under moderate load in softer gel. (F) APD alternans developed in the cell contracting under higher load in stiffer gel. (G) APD95 changes during the formation of hydrogel that progressively increase the mechanical load on the cell.

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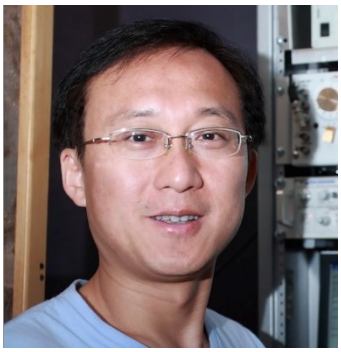
He publishes on the field of cardiac physiology and pharmacology with a focus on cellular electrophysiology, cellular calcium homeostasis and cardiac arrhythmogenesis. He has been involved in multiple international collaborative research projects in the field. He authors 132 research papers and 4 book chapters related to his research activity that resulted in more than 2000 independent citations. He is Editorial Board member at Frontiers in Physiology (EISSN 1664-042X) and served as reviewer on more than 20 journals. He is lecturer of Physiology at Faculties of General Medicine, Dentistry and Pharmacology at University of Debrecen and program coordinator of several courses. He co-authored 15 university textbooks.



Zhong Jian, Ph.D. with a multidisciplinary education and training background in semiconductor physics (B.S), statistical physics (M.S), and biophysics (Ph.D.), Zhong Jian has established a strong foundation in interdisciplinary research and expertise, particularly in the realm of cardiovascular disease mechanisms.
His research has been devoted to investigating the cellular signaling mechanisms underlying cellular mechanosensitivity and the resulting pathological consequences. His studies have encompassed various areas, such as exploration of neuronal dynamics in chronic compression-induced pathological pain, examination of TRPC channel gating properties and their mechanosensitivity, investigation of the ionic mechanisms of arrhythmia, and exploration of Ca2+ signaling in cardiac excitation-contraction coupling relevant to heart disease mechanisms. In particular, over the past decade, he has focused specifically on studying mechano-chemo-electro transduction (MCET), which aims to understand the feedback from contraction to the Ca2+ signaling system and electrical system in cardiomyocytes. To achieve this, he has developed innovative technologies such as the patch-clamp-in-gel technique and in vivo cannulation methods for isolating cardiomyocytes from heart disease models.

Bence Hegyi, MD, PhD, is currently a research scientist at the Department of Pharmacology, University of California, Davis. His research focuses on studying various molecular and cellular mechanisms responsible for the development of cardiac arrhythmias and contractile dysfunctions in heart failure and diabetes with a clear scope of linking molecular alterations to disease and translating basic research findings to clinics. He received a rigorous training in state-of-the-art electrophysiology and fluorescent imaging techniques (under the mentorship of Drs. Ye Chen-Izu and Donald M. Bers during his postdoc period), and successfully combined these -individually challenging- techniques to better understand the feedback interactions between the electrical, the Ca2+ signaling, and the contractile systems in cardiomyocytes under physiological conditions. He also significantly contributed to establish and develop several animal models of diabetic cardiomyopathy and heart failure, including new translational mouse models for heart failure with preserved ejection fraction (HFpEF), to study mechanisms of cardiac structural and transcriptional remodeling, cardiomyocyte Ca2+ signaling, contraction, and electrophysiology. Outside the lab, he is an avid nature photographer and birdwatcher.

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