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RESEARCH LETTER

Mechanical Load Regulates Excitation-Ca^{2+} Signaling-Contraction in Cardiomyocyte

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The heart is a smart pump, automatically adjusting its contractile strength in response to mechanical loads placed upon it, via intrinsic adaptations discovered over a century ago.[1,2] When the cardiomyocyte encounters an increase in preload (larger end-diastolic volume), the Frank-Starling effect enhances contractile force and stroke volume, mainly by instantaneous sarcomere-length and myofilament-based effects, independent of Ca^{2+} transient changes. In contrast, when the heart pumps blood against an increase in afterload (larger resistance), the Anrep effect develops over minutes to increase Ca^{2+} transients and enhance contractility. Most prior studies have used stretching methods to control preload on cardiomyocytes; it has been difficult to independently control afterload, and the mechanisms underlying the Anrep effect remain unresolved.[2] We have developed a new methodology to control afterload at the single-myocyte level using our Cell-in-Gel system,[3,4] and studies how afterload affects the myocyte excitation-Ca^{2+} signaling-contraction (E-C) coupling (Figure [A]).

We embedded freshly isolated rabbit ventricular myocytes in a 3-dimensional viscoelastic hydrogel comprising polyvinyl alcohol (PVA) and 4-boronate-polyethylene glycol crosslinker (Figure [B]).[4] Since the myocytes are embedded at slack length without preload, the Cell-in-Gel system is well suited for studying afterload effects. Mechanical analyses show that the myocyte contracting in-gel experiences 3-dimensional mechanical stresses including longitudinal tension due to cell shortening, transverse compression due to cell broadening, and surface traction with normal and shear stress (Figure [C]).[3]

We further developed a Patch-Clamp-in-Gel technique (Figure [D]a) using a gel-forming protocol. First, we establish patch-clamp of the cell under load-free condition with the myocyte bathed in a modified Tyrode solution containing PVA. Next, add 4-boronate-polyethylene glycol to crosslink PVA, which embeds the cell-electrode assembly in-gel. Finally, repeat the electrophysiology recordings on the same cell now contracting under afterload in-gel. Figure [D]b shows the action potentials recorded first in load-free and then under afterload. Upon adding 10% 4-boronate-polyethylene glycol to 10% PVA, polymerization occurred in only minutes. As the hydrogel became stiffer, the increases of afterload during cell contraction caused progressive increases in action potential duration (Figure [D] c). After reaching steady state, afterload significantly prolonged APD_{95} (Figure [D]d), with unchanged action potential amplitude (Figure [D]e).

We studied afterload effects on Ca^{2+} signaling and cell contraction using a gel-dissolve protocol (Figure [E]). First, we measured cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) and sarcomere length shortening simultaneously while the myocyte was embedded in-gel and paced to perform E-C coupling under afterload. Next, sorbitol (1% w/v) was added to the perfusion solution to dissolve the hydrogel. Finally, we repeated experiments on the same cell, now contracting in load-free condition. These self-control experiments show that

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Nonstandard Abbreviation and Acronyms

PVA	polyvinyl alcohol

afterload did not alter diastolic $[Ca^{2+}]_i$ (Figure [E]b) but increased systolic Ca^{2+} transients (Figure [E]c), reduced sarcomere shortening magnitude (Figure [E]c), and slowed relaxation (Figure [E]e). Thus, afterload-induced mechano-chemo-transduction regulates the Ca^{2+} signaling system, causing mechano-chemo-transduction-Ca^{2+} gain to enhance contractility.

The afterload effects on electrophysiology and Ca^{2+} signaling provide feedback loops in the dynamic system of E-C coupling, which may enable autoregulation. To test this hypothesis, we systematically tuned afterload levels using hydrogels of different stiffness (mixing 10% PVA with different crosslinker concentrations, CL%). Cardiomyocytes showed progressively larger Ca^{2+} transients under higher afterload in stiffer gels (Figure [F], CL 5%–10%, gel elastic shear modulus 1–10 kPa). Remarkably, the increases of Ca^{2+} transients enabled cardiomyocytes to maintain relatively stable contraction amplitude despite load increases (bottom). It was not until very high afterload (CL 11%–15%, gel elastic shear modulus 11–15 Pa) that myocyte contraction declined with reduced mechano-chemo-transduction-Ca^{2+} gain. In conclusion, our studies reveal mechano-chemo-electro-transduction feedbacks in the dynamic system of cardiac excitation-Ca^{2+} signaling-contraction coupling, which enable autoregulation of contractility at the single-myocyte level independent of neurohormonal influences. The Cell-in-Gel methodology provides a powerful tool for further dissection of mechano-chemo-electro-transduction molecular pathways that underlie the heart's intrinsic adaptive responses to mechanical loading in health and diseases.

ARTICLE INFORMATION

Data Availability

The methods, data, and materials of this study are available upon request to the corresponding author and through the university material transfer agreement. New Zealand White rabbits, 4 to 6 months old male, were purchased from Charles River Laboratories (Wilmington, MA) and used to isolate cardiomyocytes using standard enzymatic technique. All animal procedures were performed in strict accordance with the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health and the protocols approved by the University of California Davis Institutional Animal Care and Use Committee (IACUC).

Affiliations

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Disclosures

None.

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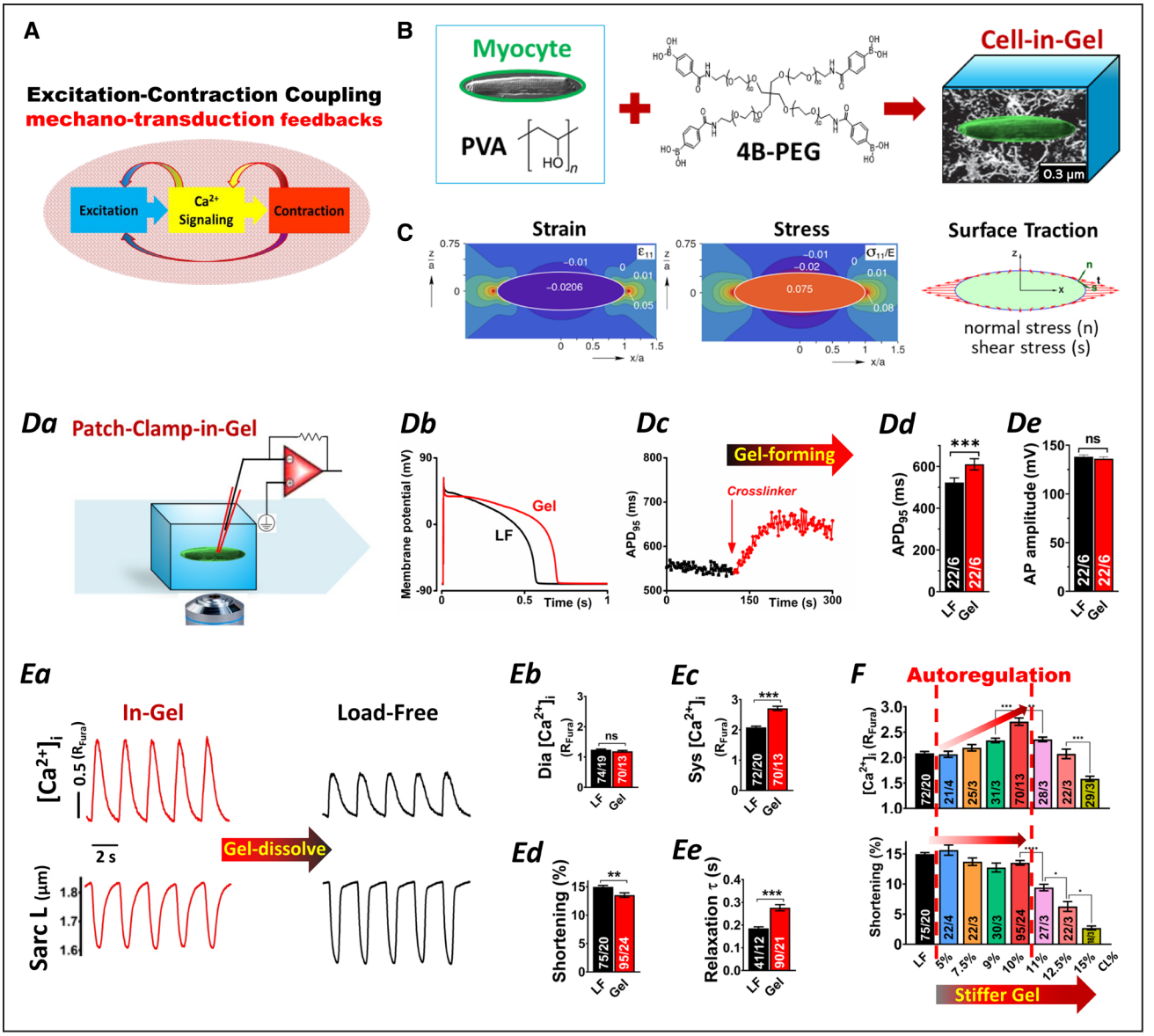

Figure. Cell-in-Gel Technology for Controlling Mechanical Load on Cells.
A, Schematic of cardiomyocyte excitation-contraction coupling with mechano-transduction feedback. **B**, Cardiomyocytes were embedded in the polymer matrix made of polyvinyl alcohol (PVA) and 4B-PEG crosslinker. **C**, Mathematical modeling of the Cell-in-Gel system show that the single cardiomyocyte experiences 3-dimensional mechanical stresses during auxotonic contraction in the hydrogel.[3] **D**, Patch-Clamp-in-Gel was performed by embedding the cell-electrode in the gel (**Da**). Action potentials (APs) were recorded first when the cell was contracting load-free (LF, black trace in **Db**), during the gel-forming protocol by adding 10% crosslinker to polymerize PVA (**Dc**), and after gel formation (Gel, red trace in **Db**). Bar charts compare cells under load-free vs in-gel, showing steady-state APD_{95} (**Dd**, $P=1.9\times10^{-10}$) and AP amplitude (**De**, $P=0.45$). **E**, Simultaneous measurements of $[Ca^{2+}]_i$ and contraction using the gel-dissolve protocol (**Ea**). Bar charts compare the cells in load-free vs in-gel, showing diastolic $[Ca^{2+}]_i$ (**Eb**, $P=0.17$), systolic peak $[Ca^{2+}]_i$ (**Ec**, $P=1.2\times10^{-12}$), contraction amplitude (**Ed**, $P=3.7\times10^{-3}$), and relaxation time (**Ee**, $P=2.12\times10^{-4}$). **F**, Cells were embedded in the gels of different stiffness by mixing 10% PVA with crosslinker of indicated concentrations (CL%). Upper shows $[Ca^{2+}]_i$ transient peak, and lower shows contraction amplitude. One-way ANOVA $P<0.0001$, and Tukey test for pair-wise comparison of neighboring groups. Statistical tests: the bars show mean and SEM of each group with indicated number of cells/animals. All groups passed D'Agostino-Pearson normality test. One-way ANOVA test was used for multiple groups comparison; *t* test for 2 groups comparison. ns indicates not significant. *P*: *$P<0.05$, **$P<0.01$, ***$P<0.001$.