Differential responses of induced pluripotent stem cell-derived cardiomyocytes to anisotropic strain depends on disease status

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Abstract

Primary dilated cardiomyopathy (DCM) is a non-ischemic heart disease with impaired pumping function of the heart. In this study, we used human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) from a healthy volunteer and a primary DCM patient to investigate the impact of DCM on iPSC-CMs' responses to different types of anisotropic strain. A bioreactor system was established that generates cardiac-mimetic forces of 150 kPa at 5% anisotropic cyclic strain and 1 Hz frequency. After confirming cardiac induction of the iPSCs, it was determined that fibronectin was favorable to other extracellular matrix protein coatings (gelatin, laminin, vitronectin) in terms of viable cell area and density, and was therefore selected as the coating for further study. When iPSC-CMs were exposed to three strain conditions (no strain, 5% static strain, and 5% cyclic strain), the static strain elicited significant induction of sarcomere components in comparison to other strain conditions. However, this induction occurred only in iPSC-CMs from a healthy volunteer ("control iPSC-CMs"), not in iPSC-CMs from the DCM patient ("DCM iPSC-CMs"). The donor type also significantly influenced gene expressions of cell-cell and cell-matrix interaction markers in response to the strain conditions. Gene expression of connexin-43 (cell-cell interaction) had a higher fold change in healthy versus diseased iPSC-CMs under static and cyclic strain, as opposed to integrins α -5 and α -10 (cell-matrix interaction). In summary, our iPSC-CM-based study to model the effects of different strain conditions suggests that intrinsic, genetic-based differences in the cardiomyocyte responses to strain may influence disease manifestation *in vivo*.

1. Introduction

Primary dilated cardiomyopathy (DCM) is a leading cause of heart failure, second only to ischemic heart disease (Sehnert et al., 2002; Sun et al., 2012). A common pathophysiology of primary DCM is thought to involve compromised contractility of cardiomyocytes (CMs), leading to impaired systolic function of the heart. As heart failure progresses, the ventricles remodel, dilate and their walls thin (Marian and Roberts, 1994; Morita et al., 2005; Willott et al., 2010). Although primary DCM is classically considered idiopathic, a significant fraction of the cases is now known to involve specific gene mutations affecting the CM structure and function (Herman et al., 2012).

In the healthy adult heart, CMs are characterized by their interactions between neighboring cells as well as the surrounding matrix. In CMs, gap junctions are responsible for mechanical and electrical coupling between cells and action potential transmission (Christoforou et al., 2013; Simon et al., 1998; Willecke et al., 2002). In particular, among the connexin gene family that is responsible for development of gap junction in CMs (Christoforou et al., 2013; Palatinus et al., 2012), connexin-43 (Cx43) is the most abundant in the heart and ventricular muscle, and low levels of its protein expression have been previously associated with ventricular arrhythmia in DCM patients (Kitamura et al., 2002; Severs et al., 2008). On the other hand, cell-matrix interactions, mediated by integrins, are also critical for the maturation and function of CMs. Of the 16 α -subunits in mammalian cells, only seven subunits, $\alpha1$, $\alpha3$, $\alpha5$, $\alpha6$, $\alpha7$, $\alpha9$, and $\alpha10$, are expressed in CMs (Ross and Borg, 2001). Fetal and neonatal CMs contain the $\alpha1$, $\alpha3$, $\alpha5$ subunits, whereas healthy adult CMs lack $\alpha1$ and $\alpha5$ subunits (Terracio et al., 1991). This suggests that these subunits can be used as markers of CM immaturity.

In order to better elucidate and ultimately regulate the cellular mechanisms that cause DCM, *in vitro* models are needed that employ a cell source recapitulating the behavior of diseased CMs. Previous studies have shown that iPSCs raised from healthy and diseased donors can be used to generate *in vitro*

models of DCM in iPSC-derived CMs (iPSC-CMs)(Rajala et al., 2011; Schwartz et al., 1995). In this study, CMs were derived from iPSCs of both a healthy donor and a 7 month old male patient with congenital DCM, a rare but fatal disorder. This DCM patient has no family history of the disease and its etiology is also unknown. CMs derived from iPSCs of both cell sources were exposed to three different conditions of anisotropic strain (no strain, 5% static strain, and 5% cyclic strain) to study how these different culture conditions influence the structure and function of CMs by altering cell-cell and cell-matrix interactions.

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84 **2. Materials and Methods**

85 *2.1 Reprogramming, Passaging, and Cardiac Differentiation of iPSCs.* Human skin fibroblasts were
86 obtained from either a healthy donor or a DCM patient (7 month old male) at the Vanderbilt University
87 Medical Center following appropriate consent under the guideline of an approved IRB protocol
88 (Vanderbilt #080369). Cells from only one patient with congenital DCM were obtained due to the rarity
89 of this disorder. The patient has no family history of DCM and its etiology is unknown. Human iPSC
90 lines were reprogrammed from skin fibroblasts by established methods (Aboud et al., 2014a; Okita et al.,
91 2011; Wang et al., 2015; Yang et al., 2014). Specifically, vector pEP4EO2SCK2MEN2L
92 (OCT4/SOX2/KLF4/MYC/NANOG/LIN28) and pEP4EO2SET2K(OCT4/SOX2/LT/KLF4) were co-
93 transfected into 1.0×10^6 human skin fibroblasts via nucleofection (VPD-1001 with program U-23,
94 Amaxa, MD). Transfected fibroblasts were plated directly to Matrigel-coated dishes in fibroblast
95 medium. On day one post-transfection, fibroblast medium was replaced with reprogramming medium
96 consisting of DMEM/F12 culture medium supplemented with N-2 supplement (Invitrogen), B27
97 (Invitrogen), 0.1mM nonessential amino acids, 1mM GlutaMAX, 0.1mM β -mercaptoethanol,
98 PD0325901 (P, 0.5 mM), CHIR99021 (C, 3 mM), A-83-01 (A, 0.5 mM) (all from Stemgent, San Diego,
99 CA), hLIF (L, 1000 U/ml, Millipore) and HA-100 (H, 10 mM). Culture media were refreshed every

100 other day. On day 13 post-transfection, the transfected cells were cultured with mTeSR1 to expand
101 iPSCs.

102 These iPSC lines were cultured and maintained following the previously published methods
103 (Aboud et al., 2012; Aboud et al., 2014b; Okita et al., 2011). Upon 80~90% confluence, cardiac
104 induction of these cells was conducted by the “Matrigel sandwich” method as described previously
105 (Zhang et al., 2012). The cardiac differentiated cells were maintained in RPMI/B27 without insulin until
106 day 10-11. The culture media was then changed every other day for one week with RPMI/B27 media
107 containing neither insulin nor glucose. The iPSC-CMs were maintained in this RPMI/B-27 supplement
108 until after cardiac differentiation on day 30.

109 *Characterization of iPSC-CMs.* Cardiac differentiation of iPSC-CMs was evaluated by Flow Cytometry
110 (FACS, BD FACS Aria III). $\sim 10^6$ cells were fixed in 4% paraformaldehyde and permeabilized by a
111 FACS buffer with 0.1% saponin. Troponin T mouse monoclonal antibodies (Santa Cruz Biotechnology)
112 diluted in FACS buffer (1:50) as primary antibodies and the primary IgG isotype (Donkey anti-mouse,
113 Invitrogen) as secondary antibodies were used for FACS analysis. The resulting data were analyzed
114 using FlowJo v8.5.2.

115 For immunostaining, fixed, permeabilized cells were blocked and treated with Troponin T mouse
116 monoclonal antibodies (Santa Cruz Biotechnology) and α -actinin mouse monoclonal antibodies (Sigma),
117 followed by incubation with IgG secondary antibodies and counter staining with DAPI. Slides were
118 viewed with a confocal microscope (Olympus FV-1000) and the image analysis was done with NIS-
119 Elements BR3.0 software.

120 *2.2 Fabrication of PDMS devices.* A silicone elastomer was created using a 1:10 ratio of catalyst to
121 elastomer base by mass (Sylgard 184, Dow Corning). The resulting poly-dimethyl siloxane (PDMS)
122 solution was poured into a pre-fabricated mold and degassed under vacuum for 15 minutes to remove air

123 bubbles. Large rectangular glass coverslips were clamped onto the PDMS molds to provide a flat surface
124 and the molds were cured overnight at 70°C. The following day, PDMS molds (overall thickness of 1/8
125 of an inch) were removed and placed in 70% ethanol for several days to remove any unreacted catalyst
126 and ultimately placed in a 90°C oven for 30 minutes to evaporate the ethanol. The device contains a
127 cellculture channel with two wells measuring 10 mm x 10 mm x 1 mm (L x W x H), in which the cells
128 were seeded and cultured. A fiberglass embedding technique was used to integrate actuator harnesses
129 into the stretcher device, allowing easy loading of the PDMS device onto the bioreactor system. The
130 elastic modulus of the device was measured in a parallel plate configuration on a rheometer (RA2000,
131 TA Instrument) in oscillation mode with a frequency of 1 Hz and 5% strain at 37°C.

132 *2.3 Bioreactor system:* In order to control the strain dynamics on the PDMS device, a design integrating
133 a fixed point and an opposing micro-positioning linear actuator were chosen. The actuators used in the
134 bioreactor system are PhysikInstrumente M-235.5DD high power direct drive DC actuators with a
135 ballscrew driving mechanism (PhysikInstrumente, GmbH). The actuators have a total travel length of
136 50mm. Ballscrew actuators were chosen to reduce the backlash when performing cyclic motion. Direct
137 drive DC-driven linear actuators were chosen to achieve the high speeds and accelerations necessary for
138 1 Hz cyclic motion akin to human physiology and produce smooth displacement curves. In addition,
139 these actuators provide forces of over 120 N and incremental motion of 0.5 µm with 0.5 µm
140 repeatability. The actuators are equipped with rotary encoders for feedback during the control process.
141 Control of the bioreactor system was achieved through a PCI bus card integrated into the control
142 computer and using Labview. Before bioreactor operation, the actuators were first referenced to non-
143 contact hall-effect limit switches and then moved into position to load the PDMS devices to the system.
144 A MATLAB program was used to write the motion profile instructions for the actuators and was capable
145 of writing any arbitrary waveform to a set of motion instructions for the actuators. Additional parameters

146 were tested, such as 1, 5, 7, and 10% strain (data not shown), frequency of strain (0.5 to 2 Hz using 0.5
147 increments (Figure 1), and length of exposure to strain was also extended past 48 hours (data not shown).
148 These parameters tested were based on previous works (Clements et al., 1997; Govoni et al., 2013b;
149 Hoit, 2011; Nguyen et al., 2013b; Shimko and Claycomb, 2008a; Yamamoto et al., 2001).

150 *2.4 Protein coating.* Clean and dry PDMS molds were placed in Harrick Plasma PDC-002 under vacuum.
151 The unit was set to high and the samples were then treated with plasma for 90 seconds. The molds were
152 removed and immediately coated with a pre-mixed solution of an extracellular matrix
153 protein($5\mu\text{g}/\text{cm}^2$)in serum-free medium. Four protein coatings were tested; gelatinas it increases beating
154 behavior and functions of cardiomyocytes (Koch-Schneidemann et al., 1994; Miskon et al.,
155 2009),lamininas it improves cell attachment of adult cardiomyocytes (Koch-Schneidemann et al., 1994),
156 fibronectinas it enhances the functions of cardiomyocytes derived from stem cells(Burridge et al., 2014;
157 Gandaglia et al., 2012), and vitronectinbecause it increases the adhesion of cardiomyocytes(Braam et al.,
158 2008; Michel, 2003). The resulting samples were covered with aluminum foil and left overnight on a
159 shaker plate at 4°C prior to seeding cells.

160 *2.5 Bioreactor culture.* iPSC-CMs were cultured on Matrigel for 30 days after cardiac induction and
161 seeded onto PDMS devices for 24 hours to allow for cell attachment. Cell-seeded PDMS stretching
162 devices were mounted on the custom-built cyclic bioreactor and placed in an incubator at 37°C and 5%
163 CO₂. Samples were cyclically stretched at 1 Hz, 5% length based strain for 48 hours(Clements et al.,
164 1997; Govoni et al., 2013a; Hoit, 2011; Nguyen et al., 2013a; Ruan et al., 2015; Shimko and Claycomb,
165 2008b; Yamamoto et al., 2001). Static controls were set up in a similar fashion and subject to 5% strain.
166 No-strain controls were placed in the incubator and not subjected to any strain(Chien et al., 2008; Gwak
167 et al., 2008).Samples were removed after 48 hours and prepared for specified endpoint assays.

168 *2.6 Characterization of iPSC-CM viability and morphology.* Cells on PDMS devices were stained with a
169 live/dead mammalian assay (Life-Tech) according to the manufacturer's protocol. A Nikon Eclipse Ti
170 inverted fluorescence microscope was used to image cells on the PDMS devices. For cell morphological
171 analysis, images were imported into ImageJ (NIH, Bethesda, MA) and analyzed for cell area and density.

172 *2.7 Analysis of gene expression by qRT-PCR.* RNA was first extracted using Trizol (Invitrogen) and the
173 RNeasy Mini Kit. cDNA was synthesized using the iScript cDNA synthesis kit (Bio-Rad) and run for
174 qRT-PCR using a thermocycler (Bio-Rad S1000TM) with Platinum[®] Supermix (Life Technologies). The
175 temperature cycle used for PCR went as follows: hot start for 3 minutes at 94°C; denaturation for 30
176 seconds at 94°C; annealing for 30 seconds; and extension for 1 minute at 72 °C. GAPDH was used as a
177 house keeping gene.

178 *2.8 Analysis of Immunofluorescence staining.* Fixed cells were permeabilized in PBS with 0.2% Triton
179 X-100 and blocked overnight in PBS, followed by treatment with either primary donkey anti-
180 cTNT antibodies or primary mouse anti-MLC-2v antibodies (both diluted to 1:200 in PBS). Samples
181 were then incubated with either secondary donkey anti-goat Cy3-conjugated antibodies or secondary
182 goat anti-mouse FITC conjugated antibodies (Abcam) (both diluted to 1:200 in PBS), followed by
183 counterstaining of nuclei with Hoechst. Images of the cells were taken with a Nikon Eclipse Ti inverted
184 fluorescence microscope, and all images were imported into ImageJ (NIH) for measurements of average
185 fluorescence intensity per cell.

186 *2.9 Statistical Analysis.* Prism 5 Software was used for data analysis. All results are reported as the mean
187 $\pm$ SEM. Unless otherwise noted, N=4 for biological replicates where the data was analyzed by two-way
188 analysis of variance (ANOVA). The Bonferroni post hoc test was used for comparison between groups,
189 and one-way ANOVA was used for analysis of unrelated groups. A value of $p < 0.05$ was considered to
190 be statistically significant.

3. Results

3.1 Bioreactor device characterization. A bioreactor was designed to produce anisotropic strain on iPSC-CMs in a controlled manner by operating two computer-programmable, compact micro-positioning stage linear actuators that can provide static or cyclic strains (See Fig. 1A, Videos S1, and the method section for details). In order to verify that the PDMS device used with the bioreactor system accurately mimicked the cardiac environment, the stiffness (Young's modulus) of the device was measured. The ultimate loading program (5% strain, 1 Hz, 48 hour culture) was determined based on the constraint that the force and cycle experienced by the cells stay within physiological cardiac range (150 kPa, 1 Hz). This modulus was measured to be approximately 150 kPa, which compares well with that of the adult human myocardium (Bhana et al., 2010; Engelmayr et al., 2008b; Forte et al., 2008; Jawad et al., 2008) (Fig. 1B).

3.2 Confirmation of cardiac induction of iPSC derived from healthy and DCM donors. Directed differentiation of iPSCs derived from both healthy and DCM donors, outlined in Figs. 2A and 2B, successfully yielded iPSC-CMs. Visual inspection of the iPSC-CMs 10-13 days after differentiation showed both iPSC-CMs formed beating sheets of cells. Flow cytometry analysis demonstrated 90-95% of both cell types were positive for troponin T at 15 days post differentiation (Fig. 2A), which was confirmed by clear visualization of cardiac α -actinin and troponin T expression in both cell types using immunostaining (Fig. 2B).

3.3 Effects of ECM Coating and Varying Strain Conditions on Cell Viability, Area, and Density. To determine an optimal extracellular matrix type for coating of the stretcher, PDMS devices were coated with gelatin, laminin, vitronectin, or fibronectin. Healthy iPSC-CMs were seeded on the PDMS devices that were either unstrained or exposed to 5% static strain, and 5% cyclic strain. Cell viability (Fig. 3C), viable cell area (Fig. 2D), and cell density (Fig. 2E) results show significant differences between the

214 types of coating. There was a significant decrease($p<0.05$) in cell viability of unstrained iPSC-CMs on a
215 vitronectin coat.No other significant differences were observed between the groups.Under all three strain
216 conditions, cells placed on fibronectin presented the largest amounts of viable cell area.Cyclic strain data
217 showed that while there was a significant increase ($P<0.05$) in viable cell density of cells on gelatin
218 compared to laminin and vitronectin, those plated on fibronectin showed the largest amounts of viable
219 cell density ($P<0.001$) compared to the other three groups.These results suggest fibronectin as the best
220 coating material among the tested ones for further experiments.

221 *3.4 Expression of Cardiac Markers.* TNNT2 is the gene that codes asarcomeric cardiac protein, cardiac
222 troponin T (cTNT), andMyosin Light Chain 2 Gene (MYL2) encodes MLC-2v protein. PCR analysis of
223 TNNT2 and MYL2 expression (Fig. 3A-B) showed that under static strain,the two genes were expressed
224 significantly more($P<0.05$) in healthy CMs compared to diseased CMs. The expression levels of these
225 genes were highest under static strain among all the test conditions. There were no significant
226 differences in the expression of these two genes between healthy and diseased CMs when unstrained and
227 exposed to cyclic strain. There were also no significant differences in the express of TNN2 between
228 diseased CMs subject to different strain types, butin diseased CMs, static and cyclic strain degreased
229 MYL2 expression significantly ($P<0.05$) compared to no strain, indicating that DCM iPSC-CMs may
230 not be healthy enough to exert positive responses to the strain conditions.

231 Protein expression of cTNT and MLC-2v (Fig.3 C-D) indicate that under static strain, these two
232 proteins were expressed significantly more in healthy CMs compared to diseased CMs ($P<0.05$), which
233 are consistent with the results of gene expression (Fig.3A-B). There were no significant differences in
234 protein expression of cTNT and MLC-2v between healthy and DCM iPSC-CMs when exposed to cyclic
235 and no strain.

236 *3.5 Cell-Cell and Cell-Matrix Interaction.* The difference in protein expression of cTNT between
 237 healthy and DCM iPSC-CMs under the static strain condition was confirmed visually by
 238 immunostaining in Fig.4A. Moreover, healthy iPSC-CMs showed aggregated cell clusters over the
 239 culture area, which was not seen in DCM iPSC-CMs. This difference indicates that altered cell-cell and
 240 cell-matrix interactions of healthy versus diseased iPSC-CMs might play a role in regulating their cardiac
 241 structure and function. Therefore, gene expression levels of connexin-43 (cell-cell interaction) and
 242 integrins $\alpha 5$ and $\alpha 10$ (cell-matrix interaction) were examined by RT-PCR (Maitra et al., 2000; Palatinus
 243 et al., 2012). The three strain conditions showed 2.4-17.3 ratio of healthy to disease (H:D) connexin-43
 244 expression (>1) (Fig.4B), indicating an enhanced cell-cell interaction in healthy over DCM iPSC-CMs.
 245 On the other hand, expression of integrins $\alpha 5$ and $\alpha 10$ showed 0.07-0.64 H:D ratio (< 1), indicating an
 246 enhanced cell-matrix interaction in the diseased over healthy cells under the three strain conditions (Fig.
 247 4C-D). While all strain groups show a substantial H:D ratio change between the expression of connexin-
 248 43 and both integrin subunits, the most notable changes are observed in the static condition. Cells
 249 exposed to cyclic strain also showed a relatively large change in H:D value between Cx43 and the
 250 integrin subunits, while a much smaller difference was observed in those that experienced no strain.
 251 Considering the fact that the H:D ratios of integrins $\alpha 5$ and $\alpha 10$ expression are similar for the three strain
 252 conditions, these results suggest the enhanced cell-cell interaction under the static and cyclic strain
 253 conditions as a critical aspect in maintaining cardiac structure and function of healthy iPSC-CMs over
 254 diseased cells.

255

256 **4. Discussion**

257 The goal of this study is to determine effects of genetic backgrounds carried from healthy and
 258 DCM patients on iPSC responses to anisotropic mechanical strain. In particular, the causative roles of

259 DCM and mechanostimulation in expression of sarcomere structure and cell-cell/cell-matrix interaction
260 markers were investigated. Either healthy or DCM iPSC-CMs we used in this study were isolated on day
261 30 after cardiac differentiation in order to keep identical maturity for all experiments. Additionally, the
262 metabolic selection method was used for purifying CMs in the iPSC-derived cell population. It was
263 expected that these minimized the variations resulted from each derivative.

264 Our study indicates that healthy and DCM iPSC-CMs show selective responses to different types
265 of strain. It was found that local increases in stationary mechanical strain (~ 150 kPa) might help
266 maintenance of cardiac structure and function of healthy iPSC-CMs because this value is similar to the
267 range of the adult human myocardium (Engelmayr et al., 2008b; Forte et al., 2008; Jawad et al., 2008).
268 However, this effect was not the same for DCM iPSC-CMs, most likely due to an altered genetic
269 background. Results from this study suggest the dominant role of cell-cell interaction in driving the
270 healthy iPSC-CM phenotype. In the analysis of matrix coatings, fibronectin proved to be the most
271 suitable for this experiment in terms of viable cell area and cell density.

272 The measured Young's modulus value of PDMS device (~ 150 kPa) matches the stiffness values
273 in the previously studied cardiac tissue models, and native rat myocardium has a Young's modulus in the
274 range of 10 to 150 kPa (Bhanna et al., 2010; Engelmayr et al., 2008a; Jawad et al., 2007). This suggests
275 that the fabricated PDMS devices are able to create a mechanical environment similar to that of heart
276 tissue. Static strain seemed to promote the healthy phenotype of iPSC-CMs, as evidenced by
277 increased expression of genes and proteins associated with cardiac structure and function. Studies with
278 mouse models have demonstrated that cTNT deficient CMs failed to assemble sarcomere structures from
279 thick and thin filaments and were not able to produce heartbeats (Ameen et al., 2008). Higher expression
280 of this protein in healthy iPSC-CMs under static conditions indicates a preferential differentiation toward
281 a healthier CM phenotype with an overall increase in contractile function of the heart. DCM iPSC-CMs,

282 on the other hand, did not respond to static strain in the same way that healthy iPSC-CMs did. MLC2
283 expression is also related to contractility of the heart, as well as thick-filament stabilization. The
284 increased expression of MLC2 gene and protein under healthy, static conditions can explain a critical
285 symptom of DCM, most notably a diminished systolic function accompanied by an inability to generate
286 the necessary contractile force. Therefore, this result supports the idea that CM structure and function are
287 affected by a pathological status (altering cellular mechanism) related to DCM (Marian and Roberts,
288 1994; Morita et al., 2005; Willott et al., 2010). A previous study reported reduced beating rates and
289 contractility with altered sarcomere structures in iPSC-CMs derived from DCM patients (Sun et al.,
290 2012). Our study provides additional insight into differences in cardiac-specific structural organization
291 and cell-cell/cell-matrix interactions between normal and DCM iPSC CMs in response to different strain
292 types.

293 Next, the effects of DCM on cell-matrix and cell-cell interactions under the three types of strain
294 were studied. Connexin-43 expression, the principle protein in gap junction interactions, was much
295 higher in healthy iPSC-CMs than in DCM iPSC-CMs in all three stretching conditions. Reduction in
296 Cx43 expression accompanies human ventricular disease and correlates with arrhythmic changes and
297 contractile dysfunction in animal models (Severs et al., 2008). Cx43 expression is expected to decrease as
298 lowered cell-cell interaction results in diminished electrical and mechanical coupling and thus a less
299 cohesive and contractile tissue. DCM cells, suffering from the inability to generate an adequate
300 contractile and uniform force, show a greatly reduced expression of this vital protein. In contrast,
301 expression of specific cell-matrix promoting proteins (integrins $\alpha 5$ and $\alpha 10$) was significantly higher in
302 iPSC-CMs derived from DCM patients. In fetal CMs, integrins $\alpha 5$ expression increases to improve cell
303 attachment to ECM components such as collagen type I and fibronectin, whereas in adult CMs, its
304 decreased expression results in reduced production of fibronectin receptors (Maitra et al., 2000). This

305 result indicates that DCM-derivedCMs may have more cell-matrix interactive, fetal phenotypes as
306 evidenced by the increased expression of ITGA5.

307 Future studies will aim to further elucidate the effects of static strain on cardiac phenotype and
308 cell-cell communication following damage from dilated cardiomyopathyusing electrophysiological tools.
309 Understanding the maturation mechanism of DCM iPSC-CMs at molecular, cellular, and physiological
310 levels willultimately lead to future development of DCM patient-specificcell therapy.

311

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463 **Figure Captions**

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465 **Figure 1.** (A) Top and side view of PDMS stretcher device. Arrow: stretching direction. (B) Force that
466 cells experience/cm² in the stretcher device. Arrow: 150 kPa at 1 Hz and an approximate 5% strain.

467 **Figure 2.** Characterization of healthy and DCM iPSC-CMs by (A) flow cytometry for expression of
468 cardiac Troponin T with corresponding healthy and DCM iPSC-CM sheets (scale bars are 100 μm) and (B)
469 immunostaining for expression of Troponin T and α-actinin. Evaluation of ECM coating materials under
470 strain by (C) cell viability, (D) cell area, and (E) cell density of healthy iPSC-CMs. * P<0.05, † P<0.01,
471 ‡ P<0.001, scale bars = 20 μm

472 **Figure 3.** Expression of cardiac markers in healthy and DCM iPSC-CMs under different mechanical
473 stimulation conditions. (A) TNNT2 gene, (B) MYL2 gene, (C) Cardiac Troponin T protein and (D)
474 MLC-2v protein. Expression of these markers was highest in healthy iPSC-CMs under 5% static at the
475 levels of both gene and protein. * P<0.05 vs. the line paired group; ‡ P<0.05 vs. the other test groups; ξ
476 P<0.05 vs. the test groups of the same cell type.

477 **Figure 4.** Cell-cell interaction of healthy iPSC-CMs was increased by mechanical stimulation. (A)
478 Immunofluorescence images of cardiac Troponin T expression in healthy and DCM iPSC-CMs under 5%
479 static condition. Gene expression ratios (Healthy: Disease=H:D) of (B) connexin 43, (C) integrin alpha 5
480 (ITGA5), and integrin alpha 10 (ITGA10) in iPSC-CMs under each condition. Fold changes normalized
481 by GAPDH. * p<0.05, comparison between healthy iPSC-CMs and DCM iPSC-CMs under the same
482 mechanical stimulation. Scale bar = 200 μm. Dashed line at a fold change ratio of 1 in all plots.

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Figure. 1

Figure 2

Figure 3

Figure 4