Mechanisms of ventricular arrhythmias elicited by coexistence of multiple electrophysiological remodeling in ischemia: a simulation study

Cuiping Liang¹, Qince Li^{1,2*}, Kuanquan Wang¹, Yimei Du⁵, Wei Wang¹, Henggui
Zhang^{1,2,3,4}

¹ School of Computer Science and Technology, Harbin Institute of Technology (HIT), Harbin 150001, China.

² Peng Cheng Laboratory, Shenzhen 518055, China.

³ School of Physics and Astronomy, The University of Manchester, Manchester M13 9PL, UK.

⁴Key Laboratory of Medical Electrophysiology of Ministry of Education and Medical Electrophysiological Key
Laboratory of Sichuan Province, Institute of Cardiovascular Research, Southwest Medical University, Luzhou,
China.

⁵Wuhan Union Hospital, Tongji Medical College of Huazhong University of Science and Technology, Wuhan,
China.

* Corresponding author: qinceli@hit.edu.cn

Abstract

Myocardial ischemia, injury and infarction (MI) are the three stages of acute coronary
syndrome (ACS). In the past two decades, a great number of studies focused on
myocardial ischemia and MI individually, and showed that the occurrence of reentrant
arrhythmias is often associated with myocardial ischemia or MI. However,
arrhythmogenic mechanisms in the tissue with various degrees of remodeling in the
ischemic heart have not been fully understood. In this study, biophysical detailed
single-cell models of ischemia 1a, 1b, and MI were developed to mimic the
electrophysiological remodeling at different stages of ACS. 2D tissue models with
different distributions of ischemia and MI were constructed to investigate the
mechanisms of initiation of reentrant waves during the progression of ischemia.

Simulation results in 2D tissues showed that the vulnerable window (VW) in the tissue with multiple pathological conditions were determined by the VWs in the tissues with a single pathological condition. In different pathological conditions, action potential duration (APD) and the conduction velocity (CV) change differently. In the tissue with multiple pathological conditions, when the borders of different pathological conditions were perpendicular to the excitation wavefront, reentrant waves were mainly induced by the spatial heterogeneity of refractory periods due to the interaction of APD and CV along the wavefront. When the borders were parallel to the wavefront, the increased excitation threshold of MI region as well as the impaired excitability of ischemia region were the primary reason for the generation of reentry. Finally, the reentrant wave was observed in a 3D model with a scar reconstructed from MRI images of a MI patient. These comprehensive findings provide novel insights for understanding the arrhythmic risk during the progression of myocardial ischemia and highlight the importance of the multiple pathological stage in designing medical therapies for arrhythmia in ischemia.

Keywords: Ventricular tachycardia, Myocardial ischemia, Myocardial infarction, Computational simulation, Reentry waves.

Author summary

Abnormal initiation or conduction of electrical impulses may lead to cardiac arrhythmias, which are very important cause of sudden and early death in developed countries. In many cases, cardiac arrhythmias are accompanied by ventricular

47 fibrillation sustained by re-entry. The occurrence of reentrant arrhythmias is often
 48 associated with acute coronary syndrome, including three phases of myocardial
 49 ischemia 1a, 1b, and infarction. Previous studies have made lots of efforts to unravel
 50 the mechanisms of the initiation and maintenance of reentry waves during myocardial
 51 ischemia or infarction. However, the mechanisms of initiation of reentrant waves in
 52 the tissue with multiple ischemic remodeling are not fully understood. Multi-scale
 53 computational models at the cellular, tissue, and organ levels were developed in this
 54 study. The main finding in this study is that reentrant waves in multiple pathological
 55 tissues were mainly induced by the spatial heterogeneity of refractory periods, when
 56 the borders of different pathological conditions were perpendicular to the wavefront
 57 of the excitation wave. In addition, the increased excitation threshold of MI region as
 58 well as the impaired excitability of ischemia region can induce the generation of
 59 reentry, when the borders were parallel to the excitation wavefront. This provides
 60 insights into mechanisms of ventricular arrhythmias elicited by coexistence of
 61 multiple electrophysiological remodeling in ischemia.

62 Introduction

63 During the progression of coronary artery stenosis or ACS, differential
 64 electrophysiological remodeling in ion currents was observed. For example, in the
 65 stage 1a of ischemia (vascular obstruction <15min), I_{Na} decreased and activation
 66 delayed, I_{NaL} increased and I_{CaL} decreased, resulting in the impairment of the
 67 excitability of cell and the decrease of APD. In the stage 1b of ischemia (vascular

68 obstruction 15~45 min), I_{CaL} further decreased, and I_{NaCa} and I_{NaK} were also inhibited,
69 resulting in the further decrease of APD. The long-lasting ischemia (vascular
70 obstruction up to 3~5days; referred as MI stage in this study) produced the largest
71 APD in these three phases due to the decline of I_{Kr} and I_{Ks} , although I_{Na} and I_{CaL} were
72 still inhibited to some extent in MI (see Table 1, Fig 2, and S1 Fig for details). At a
73 certain stage, differential electrophysiological remodeling can coexist in the same
74 heart tissues[1, 2]. Previous studies have put numerous efforts to unravel the
75 mechanisms responsible for the initiation and maintenance of reentry waves at both
76 single-cell level and tissue level under the condition of single electrophysiological
77 remodeling. For example, for stage of ischemia 1a, Kazbano *et al.* studied the effects
78 of each single component (hyperkalemia, hypoxia, and acidosis) of ischemia on
79 reentry wave propagation[3]. In addition, the inhomogeneous distribution of ion
80 currents[4] and cross wall heterogeneity[5] in ischemia 1a will enhance the
81 vulnerability to reentry. For the MI stage (i.e., the long-lasting ischemia), the changes
82 of electrophysiological characteristics and the increased heterogeneous distribution of
83 scar tissues will facilitate the generation and maintenance of reentrant waves[6, 7].
84 However, the combined effect of multiple myocardial ischemic stages on the
85 development of reentrant arrhythmias is rarely investigated.

86 In addition to the ionic remodeling, the spatial heterogeneity was shown as a key
87 factor of inducing reentry waves in myocardial ischemia or MI. In 2D regional
88 ischemic tissues[4], Romero *et al.* studied effects of spatial heterogeneity distribution
89 of ion currents and ion concentration on reentry wave propagation, and showed that

90 the mismatch of the source–sink relationship was the ultimate cause of the
 91 unidirectional block leading to reentry, rather than the dispersion of refractoriness.
 92 Weiss *et al.* studied the effects of spatial heterogeneity distribution of endocardial
 93 (Endo), intermediate layer (M), and epicardial (Epi) cells on reentrant wave
 94 propagation, and linked features of the ECG to ionic mechanisms on ischemic
 95 tissues[5]. Several studies on MI revealed the effect of the spatial heterogeneity
 96 distribution of scar areas on the occurrence of reentry waves[8-11]. However, most of
 97 these studies focused on the heterogeneity induced by Endo, M, and Epi cells, and the
 98 impacts of spatial heterogeneity induced by differential remodeling on the reentrant
 99 waves remain unclear.

100 In order to better understanding the mechanisms of cardiac arrhythmias during
 101 evolution of coronary artery blockage, multi-scale computational models at the
 102 cellular, tissue, and organ levels based on the TP06 model were developed in this
 103 study. Simulation results in 2D tissues showed that the size of VWs was inversely
 104 proportional to the CV of the tissue, and the VWs in multiple pathological tissues
 105 were determined by the VWs in single pathological tissues. In addition, the VW of the
 106 tissue with three pathological conditions was larger than that in the tissue with
 107 ischemia 1a and 1b condition, and could be further enlarged by cell-to-cell decoupling.

108 In order to reveal the mechanisms underlying the occurrence of reentry waves in
 109 multiple pathological tissues, two different spatial distributions of
 110 electrophysiological remodeling were constructed. In one case, the borders of
 111 different pathological areas are perpendicular to the excitation wavefront, and in the

other case, the borders are parallel to the excitation wavefront. Reentrant waves were mainly induced by the spatial heterogeneity of refractory periods caused by the co-action of APD and CV along the wavefront when the spatial heterogeneity distribution is perpendicular to the excitation wavefront. When the borders were parallel to the wavefront, the elevated excitation threshold in MI region and the decline of the current transfer at gap junctions (I_{gap}) were the primary reason for the generation of reentry. Finally, the simulation results on the 3D ventricle also demonstrated that the coexistence of multiple pathological conditions is more prone to induce reentry. The findings of this study sheds light on the arrhythmogenic mechanism during the progression of coronary artery blockage, and is helpful to develop new therapeutic strategies for arrhythmias in myocardial ischemia and infarction.

Methods

Single cell models

Based on the experimental data[12, 13], single cell models of ischemia 1a, 1b and MI were developed by incorporating the electrophysiological remodeling into the TP06 model. The experimental data of electrophysiological remodeling in different pathological conditions and the corresponding modifications of parameters in the computational models were summarized as shown in Table 1. In addition, in the simulations, the value of intracellular adenosine triphosphate concentration ($[ATP]_i$) was 6.8 mM in normal condition and decreased to 4.6 mM in ischemia[14] and MI[15]

133 stages according to the experimental data. The effect of $[ATP]_i$ variation in
 134 pathological conditions on cell action potential was integrated into the model via I_{KATP}
 135 whose formula was proposed by Clayton *et al.*[16].

Table 1. the electrophysiological properties of the three stages.

Simulation	Experimental data	Species	Stage	Study
$\downarrow 11.3\%, mV_{1/2} - 1.7$	1. $I_{Na} \downarrow 77\%$, $mV_{Na} \downarrow 7mV$	1.dog		1[17];
$\uparrow 50\%$	1. $I_{Na} \uparrow$; 2. $I_{Na} \uparrow 50\%$	1.human; 2.guinea pig	2,7min	1[18]; 2[19];
$\downarrow 20\%$	1. $I_{Na} \downarrow 10\% \sim 20\%$; 2. $I_{Na} \downarrow 20\%$; 5. I_{Na}	1.human; 2-4.guinea pig; 5.rabbit	2,10min	1[20]; 2[21]; 3[22]; 4[23]; 5[24, 25];
$\downarrow 50\%$, $rV_{1/2} - 7.2$, $sV_{1/2} - 13.7mV$	1. $I_{Na} \downarrow 50\%$; 2-3. $I_{Na} \downarrow$; 4. $rV_{1/2} - 7.2 mV$, $sV_{1/2} - 13.7mV$	1-2.rabbit; 3-4.human		1[25, 26]; 2[27]; 3[28]; 4[10, 11, 29]
8mM	1. 3.3-11mM; 2. 5-7mM; 3. 10 mM; 4. 4-9 mM; 5. 4-10mM; 6. 5-14mM; 7. 5-10mM	1.pig; 2-3. 5,7.rabbit; 4. 6.guinea pig	1,7,0-8min; 2-5,0-10min; 6, 0-15min	1[30]; 2[10, 11, 31]; 3[32]; 4[18, 33]; 5[18, 19, 34]; 6[35]; 7[18-20, 36];
$\downarrow 54\%$	1. $I_{NaK} \downarrow 54\%$; 2. $I_{NaK} \downarrow$	1.chick; 2.rabbit		1[37]; 2[38];
$\downarrow 40\%$	1. 4. $I_{NaCa} \downarrow$; 2. $I_{NaCa} \downarrow 54\%$; 3. $I_{NaCa} \downarrow 30\%$	1.pig; 2-4.guinea-pig; 5.canine		1[39]; 2[40]; 3[41]; 4[42]; 5[43];
$\downarrow 50\%$	1. $I_{CaL} \downarrow 51\%$; 2-4. $I_{CaL} \downarrow$	1-3.guinea pig; 4.rat	1,20min; 4,30min	1[44]; 2[45]; 3[46]; 4[47];
$\uparrow 30\%$	1. $I_{CaT} \uparrow$	1.pig		1[48];
$\downarrow 35\%$	1. $I_{rel} \downarrow 35\%$	1.rat		1[49];
$\downarrow 29\%$	1. $I_{up} \downarrow 29\%$	1.rabbit	1,15min	1[50];
$\downarrow 21.9\%$	1. $I_{Ks} \downarrow 21.9 \pm 1.8\%$	1.guinea pig		1[51];
8mM	1. 4-10mM; 2. $[K^+]_o \uparrow$	1.swine; 2. rabbit	1-2,0-30min	1[52]; 2[53];
$\downarrow 62\%$	1. $I_{Na} \downarrow 62\%$; 2. $I_{Na} \downarrow$	1,2. dog;	1-2,5 days	1[54]; 2[55];
$\downarrow 38\%$	1. $I_{CaL} \downarrow 36\%$; 2. $I_{CaL} \downarrow 38\%$; 3. $I_{CaL} \downarrow$	1-3. dog;	1-3, 5 days	1[56]; 2[57]; 3[55];
$\downarrow 63\%$	1. $I_{CaT} \downarrow 63\%$	1.dog	1,5 days	1[58];
$\downarrow 70\%$	1. $I_{Kd} \downarrow 70\%$	1.canine	1,5 days	1[59];
$\downarrow 80\%$	1. $I_{Ks} \downarrow 80\%$	1.canine	1,5 days	1[59];
8mM	$[K^+]_o \uparrow$		1,1,5-3 days	1[15];

155 The spatial distribution of various pathological condition in 2D and 3D tissues was
 156 shown in Fig 1. In 2D idealized tissue, the regions of pathological conditions along
 157 the direction of blood flow descendent are ischemia 1a, 1b, and MI regions,
 158 successively (Fig 1Ai and Aii). In order to investigate the generation of reentry waves
 159 in multiple pathological tissues, two different spatial distributions of
 160 electrophysiological remodeling were constructed. One distribution is shown in Fig
 161 1Ai where ischemia 1a, 1b, and MI distributed horizontally and excitation waves
 162 propagated vertically. In this case, the boarders of various electrophysiological
 163 remodeling were perpendicular to the wavefront of excitation waves. On the contrary,
 164 as shown in Fig 1Aii, ischemia 1a, 1b, and MI distributed circularly in the other
 165 distribution. In this case when a stimulus was applied at the upper left corner, an
 166 excitation wave propagated circularly which is parallel to the boarders of various
 167 electrophysiological remodeling.

168 For 3D ventricular tissue model, 2D sections of the scar areas were extracted from the
 169 MRI images of a MI patient. There 2D slices were interpolated and reconstructed into
 170 a 3D scar which was then incorporated into the 3D human ventricle model according
 171 to our previous method[7]. Clinical studies have shown that obstruction of the anterior
 172 descending branch of the left coronary artery leads to ischemia of the anterior wall of
 173 the left ventricle and the interventricular septum[65, 66], which is consistent with the
 174 MI areas of this patient. Similar to the assumption of the vascular blood supply in the
 175 2D tissue simulations, the blood flow direction of the coronary artery is from top to
 176 bottom. Therefore, the reconstructed scar in the 3D ventricular tissue were equally

divided into three parts (from top to bottom: ischemia 1a, ischemia 1b, and MI) as shown in Fig 1B. The tissues outside the scar areas are normal tissues.

Fig 1. Tissue architecture of (A) 2D ideal tissues and (B) the 3D real ventricle tissue with ischemia 1a, 1b, MI and normal areas.

The calculation formula of safe factor (SF) improved by Romero *et al.* was used in this paper[67], as shown below:

$$SF = \frac{\int_A I_c dt + \int_A I_{out} dt}{\int_A I_{in} dt} \quad A \mid t \in [t_{1\%dVm}, t_{Vmax}] \quad (2)$$

$$I_{in} = \sqrt{I_{in,x}^2 + I_{in,y}^2}$$

$$I_{out} = \sqrt{I_{out,x}^2 + I_{out,y}^2}$$

where I_{in} is the axial current entering the cell, I_{out} is the axial current leaving the cell, I_c is the capacitive current and A is the integration interval, from the instant when

187 membrane potential derivative reaches 1% of its maximum ($t_{1\%dV_m}$) and to the instant
188 of membrane potential peak ($t_{V_{max}}$) during the depolarization phase.

189 **Stimulation protocols of 2D and 3D tissue models**

190 The detailed information of stimulation protocols was shown in supporting
191 information. The stimulation strength was -120 pA/pF in 2D and 3D simulations, and
192 the stimulation duration was 3ms for 2D tissues and 2ms for 3D tissues. The 2D ideal
193 tissues contained 600×600 nodes with a spatial resolution of 0.25 mm, resulting in a
194 physical size of 150×150 mm². In the 3D model, an isotropic domain composed of
195 325×325×425 nodes was used with a spatial resolution of 0.33 mm, corresponding to
196 107.25×107.25×140.25 mm³ in physical size. The standard S1-S2 protocol were used
197 to initiate reentrant waves in the tissues and measure the VWs. For the S1-S2 protocol,
198 five S1 stimuli with the interval of 1000 ms were applied on the leftmost three
199 columns of nodes before S2 stimulus, which ensures the tissue reaching a steady state.
200 S2 stimulus was applied in the upper left corner or lower left corner with the size of
201 300×300 cells. In the dynamic protocol, for the tissue in Fig 1Ai, stimuli were applied
202 on the leftmost three columns of nodes, while, for the tissue in Fig 1Aii, the stimuli
203 were applied on a circular sector at the upper left corner with a radius of 5 nodes. In
204 the 3D ventricular tissue, stimuli were applied on a small cubic tissue with the size of
205 10×10×10 nodes in intramyocardial regions.

206 The differential equations were solved using the forward Euler method with a time
207 step (Δt) of 0.02 ms. In 2D and 3D tissue models, the stability criterion and the
208 Neumann boundary conditions were used[60]. An Intel core i7-3930K 64-bit CPU

209 system was used to calculate the numerical solution, and parallel computing was
210 applied for acceleration using Gtx titan z GPU.

211 Results

212 Electrophysiological remodeling in single cell models

213 The single cell models of different pathological conditions were validated via
214 comparing the variations of electrophysiological characteristics with experimental
215 data. Fig 2 showed that the variations of electrophysiological characteristics were
216 similar in three pathological stages, such as the elevated resting potentials (RPs)
217 (caused by hyperkalemia[30-34, 53]), the shortened APD (caused by the increase of
218 I_{KATP} [68, 69]), the decreased action potential amplitudes (APAs), and the reduced
219 maximum depolarization rate (dV/dt_{max}) (caused by the decrease of I_{Na} [17]), implying
220 an impaired excitability in these conditions. For ischemia 1a stage, the APD was
221 202.04ms which is within the range of human experimental data (180-260ms)[70].
222 For the ischemia phase 1b, the APD (146.86 ms) and APA (92.65 mV) were the
223 smallest among these pathological stages, while the RP (-75.55 mV) was the highest.
224 This may result in a spatial heterogeneity of the electrophysiological characteristics
225 during the progression from stenosis to occlusion in the coronary artery. The APD in
226 the ischemia 1b decreased by about half compared with that in the normal condition,
227 which is basically consistent with the study of Pollard *et al.*[71]. In short, the
228 simulation results of ischemia 1a and 1b are consistent with the previous studies[3, 16,
229 71, 72].

230 For the MI stage, previous experimental studies have shown that the APD is shortened
231 [58, 73-76]. However, it was shown that the APD was prolonged at the MI stage in
232 many previous simulation studies[6, 10, 11, 77, 78]. Since it was reported that $[ATP]_i$
233 was greatly decreased during the MI stage[15], in this study, this effect was
234 incorporated into the model via I_{KATP} current activation induced by the decrease of
235 $[ATP]_i$, leading to a shortened APD in the MI stage. As shown in Fig 2B, compared
236 with the APD (307.08 ms) in normal situation, the APD (214.74 ms) in the MI phase
237 decreased significantly, which is consistent with the experimental data[74]. Also, the
238 APA (93.63 mV) and RP (-76.23 mV) in the MI phase were agree with experimental
239 data[73]. Notably, compared with the normal condition, the dV/dt_{max} (48.93 mV/ms)
240 decreased significantly in the MI stage and was the smallest among three pathological
241 conditions as shown in Fig 2B. In summary, the simulation results of
242 electrophysiology remodeling in three pathological conditions were consistent with
243 the experimental data, which validated the developed single models of ischemia 1a,
244 1b, and MI stages.

245

246 Fig 2. The variations of electrophysiological characteristics in (A) action potentials, several main

247 ion currents, and (B) values of APA, RP, APD₉₀ and dV/dt_{max} of single cells in normal, ischemia1a,

248 ischemia1b, and MI.

249 Vulnerability of 2D tissues to reentrant waves

250 VWs were measured in single and multiple pathological tissues to examine the effect

251 of various remodeling on the vulnerability of cardiac tissue. In the single pathological

252 tissues, the generation and maintenance of reentrant waves (S2 Fig) and the

253 corresponding VWs (Fig 3B) were investigated using the S1-S2 cross-field protocol.

254 The CV in each pathological condition decreased, comparing with that in normal

255 conditions (Fig 3A), which is consistent with the relevant clinical trial records[63, 79] .

256 Among the ischemia1a, 1b, and MI tissues, the CV in MI tissue was the lowest (31.25

257 cm/s), while the CV in ischemia 1b tissue was the highest (52.25 cm/s) as shown in

258 Fig 3A. In addition, inevitably, cell-to-cell decoupling further reduced the CVs of the

259 tissue with ischemia 1b and MI to 42.75 cm/s and 25.5 cm/s, respectively. Meanwhile,

260 compared with the VW of normal tissues (110 ms), the VWs in tissues with ischemia

261 1a, 1b, and MI alone increased to 150 ms, 140 ms, and 230 ms, respectively.

262 Cell-to-cell decoupling further enlarged the VWs of the tissue with ischemia 1b and

263 MI to 170 ms and 280 ms, respectively. These simulation results showed that the size

264 of VWs is inversely proportional to the CV of the tissue, which is consistent with

265 previous study [80].

266

267 Fig 3. (A) The CV in six different 2D homogenous tissues: normal, ischemia 1a, 1b, MI,

268 decoupled 1b and decoupled MI. (B) The VWs of 2D homogenous tissues when the S2 stimulus

269 was applied in the lower left corner using the S1-S2 protocol. (C) The VWs of 2D multiple

270 pathological tissues (i) where ischemia 1a and 1b distributed horizontally and (ii) where ischemia

271 1a, 1b, and MI distributed horizontally when the S2 stimulus was applied in the (Dark Red) lower

272 left or (Orange) upper left corner using the S1-S2 protocol.

273 Then, the reentrant waves and VWs were investigated using the S1-S2 cross-field
274 protocol in the inhomogeneous tissues with multiple pathological conditions. In the
275 tissue with two pathological conditions (ischemia 1a and 1b/decoupled 1b), the S2
276 stimulus was applied in the upper or lower left corner, which is corresponding to
277 ischemia 1a or 1b region, respectively (Fig 1Ai). When the S2 stimulus was applied in
278 ischemia 1a regions (upper left corner), the upper bound of the VW of the multiple
279 pathological tissue (440 ms) was the upper bound of the VW of the homogenous
280 ischemia 1a tissue. Meanwhile the lower bound of the VW of the multiple
281 pathological tissue (250 ms) was the lower bound of the VW of the homogenous
282 ischemia 1b tissue (Fig 3Ci, orange bars). Intriguingly, on the contrary, when the
283 stimulus was applied in ischemia 1b regions (lower left corner), the upper and lower
284 bound of the VW of the inhomogeneous tissue was the upper bound of ischemia 1b
285 tissue and the lower bound of the ischemia 1a tissue, respectively (Fig 3Ci, red bars).
286 This hypothesis still held true when cell-to-cell decoupling occurred in the ischemia
287 1b region (Fig 3Ci). Therefore, the VW of multiple pathologies tissues is determined
288 by the upper and lower bounds of VWs of single pathological tissues.

289 In the above hypothesis, when the S2 stimulus was applied in ischemia 1b regions
290 (lower left corner, Fig 4A), the lower limit of the VW of multiple pathological tissue
291 is the limit allowing upward unidirectional conduction, i.e. the lower limit of the VW
292 of the homogenous ischemia 1a tissue (Fig 4A, pacing cycle length (PCL)=290ms).
293 The upper limit of the VW of multiple pathological tissue is the limit allowing
294 bidirectional conduction of S2 stimulus, i.e. the upper limit of the VW of the

295 homogenous ischemia 1b tissue (Fig 4A, PCL=420ms). Similarly, when the S2
 296 stimulus was applied in ischemia 1a regions (upper left corner, Fig 4B), the VW is
 297 determined by the limits of downward unidirectional conduction (Fig 4B,
 298 PCL=250ms) and bidirectional conduction (Fig 4B, PCL=440ms) of S2 stimulus.
 299 In the tissue with three pathological conditions (ischemia 1a, 1b, and MI), the VWs
 300 (Fig 3Cii) were not simply determined by the above conclusion. In this case, the
 301 lower limit of the VW of the multiple pathological tissue is the limit allowing
 302 upward/downward unidirectional conduction, i.e. the lower limit of the VW of the
 303 homogenous ischemia 1b tissue (Fig 3Cii, 250ms). However, the limit allowing
 304 bidirectional conduction of S2 stimulus (the upper limit of the VW) is influenced by
 305 the excitation wave propagation across two regions (ischemia 1a and 1b or ischemia
 306 1b and MI, S3 Fig). Although the upper limit of the tissue with three pathological
 307 conditions could not be precisely determined, the VW of the tissue with three
 308 pathological conditions was larger than that in the tissue with ischemia 1a and 1b
 309 condition (Fig 3Ci and Cii).

Fig 4. Wave propagation in the 2D tissue where ischemia 1a and decoupled ischemia 1b

distributed horizontally using the S1-S2 protocol when the S2 stimulus was applied in the (A) lower left or (B) upper left corner.

Initiation of reentrant waves in 2D multiple pathological tissues

In order to reveal the mechanisms underlying the genesis of reentry waves in 2D tissues, excitation wave propagation was investigated in multiple pathological tissues with two different distributions. Firstly, the reentrant waves were investigated when the spatial heterogeneity distribution was perpendicular to the excitation wavefront. In this section, both S1 and S2 stimuli were applied to the leftmost 3-line cells to induce reentrant waves (Fig 5). Different from the previous section, using this stimulation mode, reentrant waves were induced in multiple pathological tissues with ischemia 1a, 1b, and MI, but not in homogeneous 2D tissues. The VWs of occurrence reentrant waves were explored using the S1-S2 protocol (Fig 5A). In this case, the reentrant

324 wave was induced at the border between ischemia 1b and MI regions due to the
 325 conduction block of S2 in MI area. The unidirectional conduction was accounted for
 326 the long refractory period of MI induced by the low CV in MI region (Fig 5Ai).
 327 Surprisingly, the size of VWs was the same in coupling and decoupling conditions (as
 328 shown in Fig 5Aii). This may be explained by the fact that in the cell-to-cell
 329 decoupling condition, although the CVs decreased in both ischemia 1b and MI regions,
 330 the variation of CV across their border remained unchanged, i.e., the decline in CV
 331 across the border was ~40% in coupling (52.25 to 31.25 cm/s, Fig 3A) and decoupling
 332 (42.75 to 25.5 cm/s, Fig 3A) conditions. When stimuli were applied to the leftmost
 333 3-line cells using the dynamic stimulation protocol with a pacing cycle of 250ms (the
 334 detail protocol was shown in supplemental materials), spiral wave breakup occurred
 335 in the 2D tissue (Fig 5Bi), leading to severely deformed action potentials at point P2
 336 (Fig 5Bii) and thus an arrhythmic pseudo-ECG of the tissue (Fig 5Biii).

Fig 5. (A) (i) Wave propagation in the 2D tissue where ischemia 1a, decoupled 1b, and decoupled MI distributed horizontally and (ii) corresponding VWs when the leftmost stimulation was applied using S1-S2 protocol. (B) (i) Wave propagation, (ii) action potentials of points P1, P2 and P3 and (iii) Pseudo-ECG in the 2D tissue when the leftmost stimulation was applied using dynamic stimulus protocol.

Fig 6A showed the spatial distribution of APD in the 2D tissue was perpendicular to the excitation wavefront which indicated by the line L1 in Fig 6Ai. It was shown that the largest APD gradient is located at the junction of different pathological tissues

(Fig 6Aii). The difference of APD between ischemia 1a and 1b was similar to that between ischemia 1b and MI (Fig 6B), but the decline in CV between ischemia 1b and MI was much greater than that between ischemia 1a and 1b (Fig 6C). It suggested that multiple electrophysiological remodeling of various pathological conditions produced sharp heterogeneity along the excitation wavefront (the line L1). The formation of reentry waves is mainly due to the difference of CV between ischemia 1b and MI (see the details in Discussion).

Fig 6. (A) (i) APD distribution of the first stimulation in the 2D tissue where ischemia 1a, decoupled 1b, and decoupled MI distributed horizontally when the leftmost stimulation was applied with a pacing cycle of 250ms. (ii) The maximum APD difference between each cell and its neighbors in the 2D tissue. (B) APD of all cells along the line L1 in the 2D tissue. (C) CV of all cells along the line L1.

In the other spatial heterogeneity distribution, the borders of different pathological regions were parallel to the excitation wavefront (as shown in Fig 7A). In this case,

excitation wave propagation in the 2D multiple pathological tissues was investigated using the dynamic stimulation protocol. Fig 7Bi showed the development of reentrant waves in the 2D tissue with ischemia 1a, decoupled 1b, and decoupled MI when the stimulation interval was 420ms. In this process, the excitation wave was blocked at marginal areas in MI region (Fig 7Bi, 2630 ms). With the propagation of the excitation waves along the diagonal line, the excitation site of the 6th stimulus located on the boundary between MI and normal areas (Fig 7Bi, 2800 ms) resulted in backward waves (as shown by the black arrows in Fig 7Bi, 2920 ms) and generated the reentrant waves. Then, the reentrant wave collided with the excitation wave of the 7th stimulus, hindering the continued wave propagation. This process repeated in the following stimuli (Fig 7Bi, 3350-3600ms).

Fig 7. (A) (i) The 2D tissue structure where ischemia 1a, ischemia 1b and MI distributed circularly

374 and (ii) the location of point stimulation. (B) (i) Wave propagation in the 2D tissue with ischemia
375 1a, decoupled 1b, and decoupled MI. (ii) APD of all cells along the border of the 2D tissue at the
376 5th and 6th stimulation and action potentials at points P1 and P2.

377 Different from the sharp heterogeneity along the excitation wavefront when the spatial
378 heterogeneity distribution was perpendicular to the excitation wavefront (Fig 6), S4
379 Fig showed the spatial heterogeneity of APD along the excitation wavefront was
380 trivial when the spatial heterogeneity distribution was parallel to the excitation
381 wavefront. In order to reveal the mechanisms of the genesis of reentrant waves in this
382 condition, the distribution of SF in the tissue was calculated. As shown in Fig 8, the
383 SFs along Arc1(Fig 8A) were slightly smaller at the edge of the tissue (0° and 90°) in
384 the 5th stimulation. This small variation in SF along the excitation wavefront
385 gradually diminished (as shown in Arc 2, Fig 8A), and the excitation propagated
386 normally in the 5th stimulation. However, in the 6th stimulation, the SFs at the edge
387 of the tissue along Arc1(Fig 8B) further decreased to 0.875 (<1) and, therefore, led to
388 blockage of excitation waves at the edge (as shown in Fig 7Bi, 2630ms), which
389 contributed to the backward excitation waves (Fig 8B) and induced reentry. The
390 declined SFs at the edge areas mainly resulted from the decreased I_{gap} at the edge area
391 along the excitation wavefront (Fig 8Cii).

392

393 Fig 8. The SF distribution in the 2D tissue where ischemia 1a, decoupled 1b, and decoupled MI

394 distributed circularly at the (Ai) 5th and (Bi) 6th stimulation and (Aii and Bii) the SFs of all cells

395 along Arc1 and Arc2. (C) (i) Igap distribution in the 2D tissue and (ii) Igap of all cells along the

396 arc at 6th stimulation. (D) The minimum stimulation current for tissue excitability in the 2D tissue

397 at different PCL (480ms; 1000ms) in ischemia 1a, 1b and MI conditions with point stimulation in

398 the upper left corner.

399 **Reentrant waves in 3D tissue model**

Fig 9A showed that in normal conditions, reentrant waves did not occur after S1, S2 and S3 stimulation. However, in 3D ventricular tissues with multiple pathological conditions (Fig 1B), the slowed CVs in the pathological regions resulted in a slowed and heterogeneous repolarization (as shown in Fig 9B, 560ms). When the S3 stimulus was applied, the unidirectional blockage of wave propagation occurred (as shown in Fig 9B, 600ms), which in turn triggered reentrant waves (Fig 9B, 900ms). When the reentrant waves arrived the scar areas again, wavefront breakup was induced, giving rise to more disordered reentrant waves (Fig 9B, 1800ms). The reentrant waves persistently existed within 26000ms. Notably, using the same stimulation mode, reentrant waves were induced in the tissue with ischemia 1a only, but the duration of existence was relatively short (8000 ms) as shown in S5 Fig. Moreover, no reentry wave was induced in the tissue with ischemia 1b or MI only as shown in S5 Fig. It suggested that the coexistence of multiple pathological conditions in the tissue was more prone to cardiac arrhythmias.

415 Fig 9. (A) Wave propagation in the 3D ventricular tissue in normal conditions (with stimulus
416 interval 350ms and 250ms, respectively). (B) Wave propagation in the 3D ventricular tissue with
417 the ischemia 1a, 1b, and MI (with stimulus interval 310ms and 240ms, respectively).

418 Discussion

419 With the development of ischemia, different electrophysiological remodeling occurred
420 over time. Many studies have explored the effects of electrophysiological remodeling
421 and the heterogeneous distribution of tissues on the wave propagation in the ischemia
422 [4, 81] and infarcted tissues[5, 9]. However, the mechanisms of initiation of reentrant
423 waves in the tissue with multiple ischemic remodeling are not fully understood. In this
424 paper, the mechanism of cardiac arrhythmias caused by myocardial ischemia and MI
425 during coronary artery occlusion was systematically investigated. The main findings
426 of this studies are: (1) Computational models of ischemia 1a, 1b, and MI stages were
427 developed and validated using experimental data. (2) The size of VWs was inversely
428 proportional to the CV of the tissue. In 2D tissue, the VW of multiple pathological
429 tissue was determined by the VWs of single pathologic tissues. In addition, in the
430 tissue with three pathological conditions, the VW was larger than that in the tissue
431 with ischemia 1a and 1b condition, and could be further enlarged by cell-to-cell
432 decoupling. (3) When the spatial heterogeneity distribution is perpendicular to the
433 excitation wavefront, the formation of reentrant waves is caused by the spatial
434 heterogeneity of refractory periods due to the interaction of APD and CV along the
435 wavefront. And, the heterogeneity of the CV between ischemia 1b and MI on the

436 wavefront plays a major role. (4) When the spatial heterogeneity distribution is
437 parallel to the excitation wavefront, the elevated excitation threshold in MI region
438 resulted in the decline of I_{gap} and the conduction block in edge of the tissue along the
439 excitation wavefront, producing the reentrant waves.

440 **The properties of the VW of multiple pathological tissues**

441 In multiple pathological tissue, the VW, measured using the S1-S2 cross-field protocol,
442 was different when the S2 stimulus was applied at lower or upper left corner (Fig 3C).
443 The size of VWs was determined by the boundaries of individual VWs in single
444 pathological tissue. For the S1-S2 cross-field protocol, the upper and lower
445 boundaries of the VW were determined by the time limits of bidirectional (horizontal)
446 and unidirectional (vertical) propagation of S2 stimulus, respectively. In the tissue
447 with two pathological conditions (i.e. ischemia 1a and 1b, Fig 4), S2 stimulus located
448 in ischemia 1a (upper left corner) or 1b (lower left corner) region. Assuming S2
449 stimulus was applied in 1b (lower left corner) region, the time limits of horizontal and
450 vertical propagation of S2 stimulus were the upper boundary of the VW in ischemia
451 1b tissue and the lower boundary of the VW in ischemia 1a tissue (Fig 3Ci, orange
452 bar). Similar conclusion can be derived when S2 stimulus was applied in ischemia 1a
453 (upper left corner) region. In the tissue with three pathological conditions (ischemia
454 1a, 1b, and MI), the lower boundary of the VW of multiple pathological tissue is the
455 limit allowing vertically unidirectional conduction of S2 stimulus
456 (upwards/downwards, S3 Fig), i.e. the lower limit of the VW of the homogenous
457 ischemia 1b tissue (Fig 3Cii). However, the limit allowing bidirectional conduction of

458 S2 stimulus (the upper limit of the VW) was the limit allowing horizontal propagation
 459 of S2 stimulus. In this case, the horizontal propagation of S2 stimulus was influenced
 460 by the two pathological regions (ischemia 1a and 1b or ischemia 1b and MI, S3 Fig),
 461 and, therefore, cannot be derived precisely. In addition, another conclusion was that
 462 the VWs were enlarged by cell-to-cell decoupling in the tissues with two or three
 463 pathological conditions (Fig 3C), and the VW of the tissue with three pathological
 464 conditions was larger than that in the tissue with ischemia 1a and 1b condition (Fig
 465 3C), suggesting a higher risk of arrhythmias.

466 **The mechanism of reentry initiation when the spatial heterogeneity distribution**
 467 **is perpendicular to the excitation wave front**

468 When the stimulation was applied to the leftmost side of the tissue, there was no
 469 reentry wave in the single pathological tissue with ischemia 1a, 1b, or MI alone. Also,
 470 reentry was not observed in the tissue with both ischemia 1a and 1b, but was seen in
 471 the tissue with ischemia 1a, 1b, and MI (as shown in Fig 5). The VW declined in the
 472 tissue with ischemia 1a, 1b, and MI, when ischemia 1b area was replaced with
 473 ischemia 1a area (as shown in S6 Fig). When the spatial heterogeneity distribution is
 474 perpendicular to the excitation wavefront, sharp variations in electrophysiological
 475 characteristics (APD and CV) were produced at the border between different
 476 pathological conditions along excitation wavefront (Fig 6). As the excitation wave
 477 length (WL) is calculated as the product of APD and CV, the excitability and
 478 refractoriness of the tissue at the border between different pathological conditions
 479 were altered significantly. Due to the decline in APD and CV, the WL was shortened

in ischemia 1b compared to that in ischemia 1a region (Fig 5Ai, 260ms). Meanwhile, the declined CV resulted in a slowed wave propagation. These two effects counterbalanced with each other, leading to a relatively little time difference between wave tails in ischemia 1a and 1b region (Fig 5Ai, 260ms). At the border between ischemia 1b and MI, although the WL was further shortened in MI region (Fig 5Ai, 260ms), the wave tail of MI region was significantly delayed (Fig 5Ai, 260ms) due to the sharp decline in CV (Fig 6C). The delayed wave propagation increased the refractory period in MI region, and was responsible for the initiation of reentrant waves (Fig 5). Therefore, the heterogeneity along the excitation wavefront was the main reason of reentry, when the spatial heterogeneity distribution is perpendicular to the excitation wavefront. The heterogeneity of the CV on the wavefront plays an important role. Especially, the relative difference of the CV between ischemia 1b and MI is the main determinant.

Initiation of reentrant waves in the tissue with gradient heterogeneity

In the previous section, the region-wise heterogeneity caused sharp variations in electrophysiological characteristics at the borders, which was rarely seen in real heart. Therefore, it was assumed that the variations in electrophysiological characteristics across the tissue was gradually induced with the progress of myocardial ischemia, implicating a gradient-wise heterogeneity. Reentrant waves were observed when I_{KATP} and I_{Na} were set as gradient distribution individually as shown in Fig 10 (see details in supporting information). As I_{KATP} and I_{Na} are closely associated with the APD and CV, respectively, the gradient-wise heterogeneity of I_{KATP} or I_{Na} corresponded to a

502 decreased heterogeneity of APD or CV along the wavefront, respectively. As shown in
 503 Fig 10B, although both effects led to a reduced VW, a gradient distribution of I_{Na} had
 504 a more profound effect in reducing the VW. This suggested that the heterogeneity of
 505 the CV, rather than the APD, was the main factor of the genesis of reentry, which was
 506 consistent with the conclusion in region-wise heterogeneity. Although reentry was not
 507 induced when all the remodeled parameters were set as gradient distribution, breakup
 508 of reentry waves was induced using the dynamic stimulation protocol (as shown in S7
 509 Fig), indicating the potential risk of arrhythmia.

Fig 10. (A) Wave propagation in the 2D tissue with ischemia 1a, 1b, and MI and (B) VWs when the leftmost stimulation was applied using S1-S2 protocol in four different conditions: (i) region-wise heterogeneity, (ii) with gradient-wise I_{KATP} , (iii) with gradient-wise I_{Na} , (iv) gradient-wise heterogeneity of all parameters.

The mechanism of reentry initiation when the spatial heterogeneity distribution is parallel to the excitation wavefront

When the spatial heterogeneity distribution is parallel to the excitation wavefront, there was no spatial heterogeneity along the excitation wavefront at the beginning of stimulation. The simulation results showed that I_{gap} was smaller at the edge of the tissue (Fig 8C), which may be due to less cells were coupled at the edge, leading to a weaker drive force for excitation. Moreover, it was shown that the excitation threshold in MI region was dramatically increased at high pacing frequency (Fig 8D), implicating that the drive force required for excitation in MI region was enlarged. In this case, with the fast stimulation using the dynamic stimulation protocol, the safe factor for conduction at the edge in MI region was gradually decreased (Fig 8A and 8B), which eventually gave rise to the conduction block at the edge and promote the formation of reentrant waves (Fig 7).

What's more, if the ischemia 1b area was replaced with the ischemia 1a area, the VW declined from 50 ms (420 ms~470 ms) to 40 ms (430 ms~470 ms), when the spatial heterogeneity distribution is parallel to the excitation wavefront. As shown in Figs 2 and 3, compared with ischemia 1a stage, the lower APA, dV/dt_{max} and CV as well as the higher RP impaired the excitability of ischemia 1b region, impeded the wave

533 propagation. Therefore, even the electrophysiological remodeling is homogeneous
 534 along the excitation wavefront, the axial spatial heterogeneity caused by multiple
 535 pathological conditions may hinder the wave propagation, further reduced the drive
 536 force for excitation, and contributed to the conduction block at the edge in MI region.
 537 As the edge of ischemic region in real heart tissue is much more complicated than the
 538 ideal tissue in this study, the risk of reentrant arrhythmia would be higher when
 539 multiple pathological conditions coexisted.

540 **Reentrant waves in 3D tissue**

541 Reentrant arrhythmias occurring on the ventricles are mainly ventricular tachycardia
 542 and ventricular fibrillation, and ventricular fibrillation is one of the most dangerous
 543 arrhythmias, the hallmark of which is the appearance of wave fissures[82]. In the 3D
 544 ventricular tissue, persistent reentrant waves were observed in the tissue with three
 545 pathological conditions, rather than in the single pathological tissue with ischemia 1a,
 546 1b, or MI. This phenomenon was consistent with our conclusion in the previous
 547 section that in multiple pathological tissue using the leftmost stimulation, the
 548 enhanced spatial heterogeneity of the tissue promoted the generation of reentry waves,
 549 thus greatly increased the risk of arrhythmia. In addition, the anatomical heterogeneity
 550 in real heart tissue further amplified the risk of reentrant arrhythmia in multiple
 551 pathological conditions, since it would augment the spatial heterogeneity along the
 552 wavefront as well as along the edge of ischemic region.

553 **Limitation**

554 The limitations of this paper exist in the following aspects. At single cell-level, the
 555 limitations of TP06 cell model also exist in our simulation, for example, the effect of
 556 CaMKII is not included. In addition, it depends on the further development of
 557 patch-clamp experimental studies of human cells in ischemia 1a, 1b, and MI, so that
 558 more real human data can be used to remodel single cells. In addition, the effect of the
 559 heterogeneity of the three cardiomyocyte cells (Endo, M, Epi cells) was not compared
 560 in our single-cell and 2D simulation experiments, only the Epi cells were used.
 561 However, this does not change the conclusions in single-cell and 2D tissue
 562 simulations, and these three different cells were included in our 3D real ventricular
 563 tissues. The stage of MI is often accompanied by fibrosis, while fibrosis tissues were
 564 not added into our models, which can be supplemented in the subsequent simulation
 565 work. Furthermore, more diversified and complex distribution of ischemia 1a, 1b, and
 566 MI areas may be designed in 2D tissues and 3D tissues, but this will not change our
 567 conclusions.

568 **Author Contributions**

569 Conceptualization: Qince Li, Henggui Zhang.
 570 Data curation: Cuiping Liang, Qince Li.
 571 Formal analysis: Cuiping Liang, Qince Li, Kuanquan Wang, Henggui Zhang.
 572 Funding acquisition: Yimei Du, Qince Li, Kuanquan Wang.
 573 Investigation: Cuiping Liang, Qince Li, Kuanquan Wang.

574 Methodology: Cuiping Liang, Qince Li, Henggui Zhang.

575 Project administration: Qince Li, Kuanquan Wang, Henggui Zhang.

576 Resources: Qince Li, Kuanquan Wang, Henggui Zhang.

577 Software: Cuiping Liang, Henggui Zhang.

578 Supervision: Qince Li, Kuanquan Wang, Henggui Zhang.

579 Writing – original draft: Cuiping Liang, Qince Li.

580 Writing – review & editing: Cuiping Liang, Qince Li, Kuanquan Wang, Yimei Du,

581 Wei Wang, Henggui Zhang.

582 Reference

583 1. Anversa P, Li P, Zhang X, Olivetti G, Capasso JM. Ischaemic myocardial injury and

584 ventricular remodelling. Cardiovascular research. 1993;27(2):145-57. PMID: 8472264.

585 2. Hegyi B, Bossuyt J, Griffiths LG, Shimkunas R, Coulibaly Z, Jian Z, et al. Complex

586 electrophysiological remodeling in postinfarction ischemic heart failure. Proc Natl Acad Sci U S

587 A. 2018;115(13):E3036-E44. doi: 10.1073/pnas.1718211115. PMID: 29531045.

588 3. Kazbanov IV, Clayton RH, Nash MP, Bradley CP, Paterson DJ, Hayward MP, et al. Effect

589 of global cardiac ischemia on human ventricular fibrillation: insights from a multi-scale

590 mechanistic model of the human heart. PLoS Comput Biol. 2014;10(11):e1003891. doi:

591 10.1371/journal.pcbi.1003891. PMID: 25375999.

592 4. Romero L, Trenor B, Alonso JM, Tobon C, Saiz J, Ferrero JM, Jr. The relative role of

593 refractoriness and source-sink relationship in reentry generation during simulated acute

594 ischemia. Ann Biomed Eng. 2009;37(8):1560-71. doi: 10.1007/s10439-009-9721-2. PMID:

595 19495982.

596 5. Weiss DL, Ifland M, Sachse FB, Seemann G, Dossel O. Modeling of cardiac ischemia in
597 human myocytes and tissue including spatiotemporal electrophysiological variations. *Biomed*
598 *Tech (Berl)*. 2009;54(3):107-25. doi: 10.1515/BMT.2009.016. PMID: 19469661.

599 6. Connolly AJ, Bishop MJ. Computational Representations of Myocardial Infarct Scars and
600 Implications for Arrhythmogenesis. *Clin Med Insights Cardiol*. 2016;10(Suppl 1):27-40. doi:
601 10.4137/CMC.S39708. PMID: 27486348.

602 7. Liang C, Wang K, Li Q, Bai J, Zhang H. Influence of the distribution of fibrosis within an
603 area of myocardial infarction on wave propagation in ventricular tissue. *Sci Rep*.
604 2019;9(1):14151. doi: 10.1038/s41598-019-50478-5. PMID: 31578428.

605 8. Cabo C, Boyden PA. Electrical remodeling of the epicardial border zone in the canine
606 infarcted heart: a computational analysis. *Am J Physiol Heart Circ Physiol*.
607 2003;284(1):H372-84. doi: 10.1152/ajpheart.00512.2002. PMID: 12388240.

608 9. Ringenber J, Deo M, Filgueiras-Rama D, Pizarro G, Ibanez B, Peinado R, et al. Effects
609 of fibrosis morphology on reentrant ventricular tachycardia inducibility and simulation fidelity in
610 patient-derived models. *Clin Med Insights Cardiol*. 2014;8(Suppl 1):1-13. doi:
611 10.4137/CMC.S15712. PMID: 25368538.

612 10. Arevalo H, Plank G, Helm P, Halperin H, Trayanova N. Tachycardia in post-infarction
613 hearts: insights from 3D image-based ventricular models. *PLoS One*. 2013;8(7):e68872. doi:
614 10.1371/journal.pone.0068872. PMID: 23844245.

615 11. Deng D, Arevalo HJ, Prakosa A, Callans DJ, Trayanova NA. A feasibility study of
616 arrhythmia risk prediction in patients with myocardial infarction and preserved ejection fraction.

- 617 Europace. 2016;18(suppl 4):iv60-iv6. doi: 10.1093/europace/euw351. PMID: 28011832.
- 618 12. ten Tusscher KH, Noble D, Noble PJ, Panfilov AV. A model for human ventricular tissue.
- 619 American journal of physiology Heart and circulatory physiology. 2004;286(4):H1573-89. doi:
- 620 10.1152/ajpheart.00794.2003. PMID: 14656705.
- 621 13. ten Tusscher KH, Panfilov, A.V. Alternans and spiral breakup in a human ventricular tissue
- 622 model. American journal of physiology Heart and circulatory physiology.
- 623 2006;291(3):H1088-100. doi: 10.1152/ajpheart.00109.2006. PMID: 16565318.
- 624 14. Babenko AP, Gonzalez G, Aguilar-Bryan L, Bryan J. Reconstituted human cardiac KATP
- 625 channels: functional identity with the native channels from the sarcolemma of human
- 626 ventricular cells. Circ Res. 1998;83(11):1132-43. doi: 10.1161/01.res.83.11.1132. PMID:
- 627 9831708.
- 628 15. Di Diego JM, Antzelevitch C. Ischemic ventricular arrhythmias: experimental models and
- 629 their clinical relevance. Heart Rhythm. 2011;8(12):1963-8. doi: 10.1016/j.hrthm.2011.06.036.
- 630 PMID: 21740880.
- 631 16. Clayton RH, Nash MP, Bradley CP, Panfilov AV, Paterson DJ, Taggart P.
- 632 Experiment-model interaction for analysis of epicardial activation during human ventricular
- 633 fibrillation with global myocardial ischaemia. Prog Biophys Mol Biol. 2011;107(1):101-11. doi:
- 634 10.1016/j.pbiomolbio.2011.06.010. PMID: 21741985.
- 635 17. Murphy L, Renodin D, Antzelevitch C, Di Diego JM, Cordeiro JM. Extracellular proton
- 636 depression of peak and late Na(+) current in the canine left ventricle. Am J Physiol Heart Circ
- 637 Physiol. 2011;301(3):H936-44. doi: 10.1152/ajpheart.00204.2011. PMID: 21685271.
- 638 18. Jones DK, Peters CH, Tolhurst SA, Claydon TW, Ruben PC. Extracellular proton

639 modulation of the cardiac voltage-gated sodium channel, Nav1.5. *Biophys J*.
640 2011;101(9):2147-56. doi: 10.1016/j.bpj.2011.08.056. PMID: 22067152.

641 19. Zheng J, Ma J, Zhang P, Hu L, Fan X, Tang Q. Milrinone inhibits hypoxia or hydrogen
642 dioxide-induced persistent sodium current in ventricular myocytes. *Eur J Pharmacol*.
643 2009;616(1-3):206-12. doi: 10.1016/j.ejphar.2009.06.021. PMID: 19549513.

644 20. Fernandez-Morales JC, Hua W, Yao Y, Morad M. Regulation of Ca(2+) signaling by acute
645 hypoxia and acidosis in cardiomyocytes derived from human induced pluripotent stem cells.
646 *Cell Calcium*. 2019;78:1-14. doi: 10.1016/j.ceca.2018.12.006. PMID: 30579812.

647 21. Sato R, Noma A, Kurachi Y, Irisawa H. Effects of intracellular acidification on membrane
648 currents in ventricular cells of the guinea pig. *Circ Res*. 1985;57(4):553-61. doi:
649 10.1161/01.res.57.4.553. PMID: 2412722.

650 22. Hool LC. Hypoxia increases the sensitivity of the L-type Ca(2+) current to beta-adrenergic
651 receptor stimulation via a C2 region-containing protein kinase C isoform. *Circ Res*.
652 2000;87(12):1164-71. doi: 10.1161/01.res.87.12.1164. PMID: 11110774.

653 23. Irisawa H, Sato R. Intra- and extracellular actions of proton on the calcium current of
654 isolated guinea pig ventricular cells. *Circ Res*. 1986;59(3):348-55. doi:
655 10.1161/01.res.59.3.348. PMID: 2429781.

656 24. Saegusa N, Moorhouse E, Vaughan-Jones RD, Spitzer KW. Influence of pH on Ca(2+)(+)
657 current and its control of electrical and Ca(2+)(+) signaling in ventricular myocytes. *J Gen*
658 *Physiol*. 2011;138(5):537-59. doi: 10.1085/jgp.201110658. PMID: 22042988.

659 25. Ferrero JM, Trenor B, Romero L. Multiscale computational analysis of the bioelectric
660 consequences of myocardial ischaemia and infarction. *Europace*. 2014;16(3):405-15. doi:

- 10.1093/europace/eut405. PMID: 24569895.
26. Saegusa N, Garg V, Spitzer KW. Modulation of ventricular transient outward K(+) current by acidosis and its effects on excitation-contraction coupling. *Am J Physiol Heart Circ Physiol*. 2013;304(12):H1680-96. doi: 10.1152/ajpheart.00070.2013. PMID: 23585132.
27. Ogbaghebriel A, Shrier A. Inhibition of metabolism abolishes transient outward current in rabbit atrial myocytes. *Am J Physiol*. 1994;266(1 Pt 2):H182-90. doi: 10.1152/ajpheart.1994.266.1.H182. PMID: 8304498.
28. Verkerk AO, Veldkamp MW, van Ginneken AC, Bouman LN. Biphasic response of action potential duration to metabolic inhibition in rabbit and human ventricular myocytes: role of transient outward current and ATP-regulated potassium current. *J Mol Cell Cardiol*. 1996;28(12):2443-56. doi: 10.1006/jmcc.1996.0237. PMID: 9004161.
29. Stengl M, Cameliet E, Mubagwa K, Flameng W. Modulation of transient outward current by extracellular protons and Cd²⁺ in rat and human ventricular myocytes. *J Physiol*. 1998;511 (Pt 3):827-36. doi: 10.1111/j.1469-7793.1998.827bg.x. PMID: 9714863.
30. Watanabe I, Kanda A, Engle CL, Gettes LS. Comparison of the effects of regional ischemia and hyperkalemia on the membrane action potentials of the in situ pig heart. Experimental Cardiology Group, University of North Carolina at Chapel Hill. *J Cardiovasc Electrophysiol*. 1997;8(11):1229-36. doi: 10.1111/j.1540-8167.1997.tb01012.x. PMID: 9395164.
31. Vermeulen JT, Tan HL, Rademaker H, Schumacher CA, Loh P, Opthof T, et al. Electrophysiologic and extracellular ionic changes during acute ischemia in failing and normal rabbit myocardium. *J Mol Cell Cardiol*. 1996;28(1):123-31. doi: 10.1006/jmcc.1996.0012.

683 PMID: 8745220.

684 32. Weiss J, Shine KI. [K⁺]_o accumulation and electrophysiological alterations during early
685 myocardial ischemia. Am J Physiol. 1982;243(2):H318-27. doi:
686 10.1152/ajpheart.1982.243.2.H318. PMID: 7114241.

687 33. Weiss JN, Venkatesh N, Lamp ST. ATP-sensitive K⁺ channels and cellular K⁺ loss in
688 hypoxic and ischaemic mammalian ventricle. J Physiol. 1992;447:649-73. doi:
689 10.1113/jphysiol.1992.sp019022. PMID: 1593462.

690 34. Weiss J, Shine KI. Effects of heart rate on extracellular [K⁺] accumulation during
691 myocardial ischemia. Am J Physiol. 1986;250(6 Pt 2):H982-91. doi:
692 10.1152/ajpheart.1986.250.6.H982. PMID: 3717369.

693 35. Kleber AG. Resting membrane potential, extracellular potassium activity, and intracellular
694 sodium activity during acute global ischemia in isolated perfused guinea pig hearts. Circ Res.
695 1983;52(4):442-50. doi: 10.1161/01.res.52.4.442. PMID: 6831660.

696 36. Owens LM, Fralix TA, Murphy E, Cascio WE, Gettes LS. Correlation of ischemia-induced
697 extracellular and intracellular ion changes to cell-to-cell electrical uncoupling in isolated
698 blood-perfused rabbit hearts. Experimental Working Group. Circulation. 1996;94(1):10-3. doi:
699 10.1161/01.cir.94.1.10. PMID: 8964108.

700 37. Ikenouchi H, Zhao L, McMillan M, Hammond EM, Barry WH. ATP depletion causes a
701 reversible decrease in Na⁺ pump density in cultured ventricular myocytes. Am J Physiol.
702 1993;264(4 Pt 2):H1208-14. doi: 10.1152/ajpheart.1993.264.4.H1208. PMID: 8386481.

703 38. Bersohn MM. Sodium pump inhibition in sarcolemma from ischemic hearts. J Mol Cell
704 Cardiol. 1995;27(8):1483-9. doi: 10.1016/s0022-2828(95)90161-2. PMID: 8523412.

39. Doering AE, Lederer WJ. The mechanism by which cytoplasmic protons inhibit the sodium-calcium exchanger in guinea-pig heart cells. *J Physiol.* 1993;466:481-99. PMID: 8410703.
40. Shigematsu S, Arita M. Anoxia depresses sodium-calcium exchange currents in guinea-pig ventricular myocytes. *J Mol Cell Cardiol.* 1999;31(4):895-906. doi: 10.1006/jmcc.1998.0929. PMID: 10329216.
41. Egger M, Niggli E. Paradoxical block of the Na⁺-Ca²⁺ exchanger by extracellular protons in guinea-pig ventricular myocytes. *J Physiol.* 2000;523 Pt 2:353-66. doi: 10.1111/j.1469-7793.2000.t01-1-00353.x. PMID: 10699080.
42. Earm YE, Irisawa H. Effects of pH on the Na-Ca exchange current in single ventricular cells of the guinea pig. *Jpn Heart J.* 1986;27 Suppl 1:153-8. PMID: 2434676.
43. Philipson KD, Bersohn MM, Nishimoto AY. Effects of pH on Na⁺-Ca²⁺ exchange in canine cardiac sarcolemmal vesicles. *Circ Res.* 1982;50(2):287-93. doi: 10.1161/01.res.50.2.287. PMID: 7055859.
44. Cordeiro JM, Howlett SE, Ferrier GR. Simulated ischaemia and reperfusion in isolated guinea pig ventricular myocytes. *Cardiovasc Res.* 1994;28(12):1794-802. doi: 10.1093/cvr/28.12.1794. PMID: 7867032.
45. Prod'homme B, Pietrobon D, Hess P. Interactions of protons with single open L-type calcium channels. Location of protonation site and dependence of proton-induced current fluctuations on concentration and species of permeant ion. *J Gen Physiol.* 1989;94(1):23-42. doi: 10.1085/jgp.94.1.23. PMID: 2553858.
46. Kaibara M, Kameyama M. Inhibition of the calcium channel by intracellular protons in

727 single ventricular myocytes of the guinea-pig. J Physiol. 1988;403:621-40. doi:
728 10.1113/jphysiol.1988.sp017268. PMID: 2855346.

729 47. Wang X, Wang X, Gu Y, Wang T, Huang C. Wenxin Keli attenuates ischemia-induced
730 ventricular arrhythmias in rats: Involvement of Ltype calcium and transient outward potassium
731 currents. Mol Med Rep. 2013;7(2):519-24. doi: 10.3892/mmr.2012.1195. PMID: 23174802.

732 48. Wang SY, Clague JR, Langer GA. Increase in calcium leak channel activity by metabolic
733 inhibition or hydrogen peroxide in rat ventricular myocytes and its inhibition by polycation. J
734 Mol Cell Cardiol. 1995;27(1):211-22. doi: 10.1016/s0022-2828(08)80020-x. PMID: 7760345.

735 49. Balnave CD, Vaughan-Jones RD. Effect of intracellular pH on spontaneous Ca²⁺ sparks
736 in rat ventricular myocytes. J Physiol. 2000;528 Pt 1:25-37. doi:
737 10.1111/j.1469-7793.2000.00025.x. PMID: 11018103.

738 50. Kaplan P, Hendrikx M, Mattheussen M, Mubagwa K, Flameng W. Effect of ischemia and
739 reperfusion on sarcoplasmic reticulum calcium uptake. Circ Res. 1992;71(5):1123-30. doi:
740 10.1161/01.res.71.5.1123. PMID: 1394874.

741 51. Hool LC. Differential regulation of the slow and rapid components of guinea-pig cardiac
742 delayed rectifier K⁺ channels by hypoxia. J Physiol. 2004;554(Pt 3):743-54. doi:
743 10.1113/jphysiol.2003.055442. PMID: 14634203.

744 52. Hill JL, Gettes LS. Effect of acute coronary artery occlusion on local myocardial
745 extracellular K⁺ activity in swine. Circulation. 1980;61(4):768-78. doi: 10.1161/01.cir.61.4.768.
746 PMID: 7357719.

747 53. Wilde AA, Escande D, Schumacher CA, Thuringer D, Mestre M, Fiolet JW, et al.
748 Potassium accumulation in the globally ischemic mammalian heart. A role for the

749 ATP-sensitive potassium channel. *Circ Res.* 1990;67(4):835-43. doi: 10.1161/01.res.67.4.835.
750 PMID: 2119912.

751 54. Pu J, Boyden PA. Alterations of Na⁺ currents in myocytes from epicardial border zone of
752 the infarcted heart. A possible ionic mechanism for reduced excitability and postrepolarization
753 refractoriness. *Circ Res.* 1997;81(1):110-9. doi: 10.1161/01.res.81.1.110. PMID: 9201034.

754 55. Baba S, Dun W, Cabo C, Boyden PA. Remodeling in cells from different regions of the
755 reentrant circuit during ventricular tachycardia. *Circulation.* 2005;112(16):2386-96. doi:
756 10.1161/CIRCULATIONAHA.105.534784. PMID: 16203911.

757 56. Aggarwal R, Boyden PA. Diminished Ca²⁺ and Ba²⁺ currents in myocytes surviving in
758 the epicardial border zone of the 5-day infarcted canine heart. *Circ Res.* 1995;77(6):1180-91.
759 doi: 10.1161/01.res.77.6.1180. PMID: 7586231.

760 57. Aggarwal R, Boyden PA. Altered pharmacologic responsiveness of reduced L-type
761 calcium currents in myocytes surviving in the infarcted heart. *J Cardiovasc Electrophysiol.*
762 1996;7(1):20-35. doi: 10.1111/j.1540-8167.1996.tb00457.x. PMID: 8718981.

763 58. Lue WM, Boyden PA. Abnormal electrical properties of myocytes from chronically
764 infarcted canine heart. Alterations in V_{max} and the transient outward current. *Circulation.*
765 1992;85(3):1175-88. doi: 10.1161/01.cir.85.3.1175. PMID: 1371431.

766 59. Jiang M, Cabo C, Yao J, Boyden PA, Tseng G. Delayed rectifier K currents have reduced
767 amplitudes and altered kinetics in myocytes from infarcted canine ventricle. *Cardiovasc Res.*
768 2000;48(1):34-43. PMID: 11033106.

769 60. Clayton RH, Panfilov, A.V. A guide to modelling cardiac electrical activity in anatomically
770 detailed ventricles. *Prog Biophys Mol Biol.* 2008;96(1-3):19-43. doi:

771 10.1016/j.pbiomolbio.2007.07.004. PMID: 17825362.

772 61. Smith WTt, Fleet WF, Johnson TA, Engle CL, Cascio WE. The Ib phase of ventricular
773 arrhythmias in ischemic in situ porcine heart is related to changes in cell-to-cell electrical
774 coupling. Experimental Cardiology Group, University of North Carolina. Circulation.
775 1995;92(10):3051-60. doi: 10.1161/01.cir.92.10.3051. PMID: 7586276.

776 62. De Groot JR, Coronel R. Acute ischemia-induced gap junctional uncoupling and
777 arrhythmogenesis. Cardiovasc Res. 2004;62(2):323-34. doi: 10.1016/j.cardiores.2004.01.033.
778 PMID: 15094352.

779 63. Jongsma HJ, Wilders R. Gap junctions in cardiovascular disease. Circ Res.
780 2000;86(12):1193-7. doi: 10.1161/01.res.86.12.1193. PMID: 10864907.

781 64. Cabo C, Yao J, Boyden PA, Chen S, Hussain W, Duffy HS, et al. Heterogeneous gap
782 junction remodeling in reentrant circuits in the epicardial border zone of the healing canine
783 infarct. Cardiovasc Res. 2006;72(2):241-9. doi: 10.1016/j.cardiores.2006.07.005. PMID:
784 16914125.

785 65. James TN, Burch GE. Blood supply of the human interventricular septum. Circulation.
786 1958;17(3):391-6. doi: 10.1161/01.cir.17.3.391. PMID: 13511658.

787 66. Morales AR, Romanelli R, Boucek RJ. The mural left anterior descending coronary artery,
788 strenuous exercise and sudden death. Circulation. 1980;62(2):230-7. doi:
789 10.1161/01.cir.62.2.230. PMID: 7397963.

790 67. Romero L, Trenor B, Ferrero JM, Stamer CF. Non-uniform dispersion of the source-sink
791 relationship alters wavefront curvature. PLoS One. 2013;8(11):e78328. doi:
792 10.1371/journal.pone.0078328. PMID: 24223791.

- 793 68. Ferrero JM, Jr., Saiz J, Ferrero JM, Thakor NV. Simulation of action potentials from
794 metabolically impaired cardiac myocytes. Role of ATP-sensitive K⁺ current. *Circ Res*.
795 1996;79(2):208-21. doi: 10.1161/01.res.79.2.208. PMID: 8755997.
- 796 69. Shaw RM, Rudy Y. Electrophysiologic effects of acute myocardial ischemia: a theoretical
797 study of altered cell excitability and action potential duration. *Cardiovasc Res*.
798 1997;35(2):256-72. doi: 10.1016/s0008-6363(97)00093-x. PMID: 9349389.
- 799 70. Sutton PM, Taggart P, Opthof T, Coronel R, Trimlett R, Pugsley W, et al. Repolarisation
800 and refractoriness during early ischaemia in humans. *Heart*. 2000;84(4):365-9. doi:
801 10.1136/heart.84.4.365. PMID: 10995401.
- 802 71. Pollard AE, Cascio WE, Fast VG, Knisley SB. Modulation of triggered activity by
803 uncoupling in the ischemic border. A model study with phase 1b-like conditions. *Cardiovasc*
804 *Res*. 2002;56(3):381-92. doi: 10.1016/s0008-6363(02)00598-9. PMID: 12445879.
- 805 72. Dutta S, Mincholé A, Quinn TA, Rodriguez B. Electrophysiological properties of
806 computational human ventricular cell action potential models under acute ischemic conditions.
807 *Prog Biophys Mol Biol*. 2017;129:40-52. doi: 10.1016/j.pbiomolbio.2017.02.007. PMID:
808 28223156.
- 809 73. Ursell PC, Gardner PI, Albala A, Fenoglio JJ, Jr., Wit AL. Structural and
810 electrophysiological changes in the epicardial border zone of canine myocardial infarcts during
811 infarct healing. *Circ Res*. 1985;56(3):436-51. doi: 10.1161/01.res.56.3.436. PMID: 3971515.
- 812 74. Gardner PI, Ursell PC, Fenoglio JJ, Jr., Wit AL. Electrophysiologic and anatomic basis for
813 fractionated electrograms recorded from healed myocardial infarcts. *Circulation*.
814 1985;72(3):596-611. doi: 10.1161/01.cir.72.3.596. PMID: 4017211.

815 75. Spear JF, Michelson EL, Moore EN. Cellular electrophysiologic characteristics of
816 chronically infarcted myocardium in dogs susceptible to sustained ventricular tachyarrhythmias.
817 J Am Coll Cardiol. 1983;1(4):1099-110. doi: 10.1016/s0735-1097(83)80112-0. PMID:
818 6833648.

819 76. Boyden PA, Gardner PI, Wit AL. Action potentials of cardiac muscle in healing infarcts:
820 response to norepinephrine and caffeine. J Mol Cell Cardiol. 1988;20(6):525-37. doi:
821 10.1016/s0022-2828(88)80079-8. PMID: 2851055.

822 77. Deng D, Arevalo H, Pashakhanloo F, Prakosa A, Ashikaga H, McVeigh E, et al. Accuracy
823 of prediction of infarct-related arrhythmic circuits from image-based models reconstructed from
824 low and high resolution MRI. Front Physiol. 2015;6:282. doi: 10.3389/fphys.2015.00282. PMID:
825 26528188.

826 78. McDowell KS, Arevalo HJ, Maleckar MM, Trayanova NA. Susceptibility to arrhythmia in
827 the infarcted heart depends on myofibroblast density. Biophys J. 2011;101(6):1307-15. doi:
828 10.1016/j.bpj.2011.08.009. PMID: 21943411.

829 79. Taggart P, Sutton PM, Opthof T, Coronel R, Trimlett R, Pugsley W, et al. Inhomogeneous
830 transmural conduction during early ischaemia in patients with coronary artery disease. J Mol
831 Cell Cardiol. 2000;32(4):621-30. doi: 10.1006/jmcc.2000.1105. PMID: 10756118.

832 80. Gaztanaga L, Marchlinski FE, Betensky BP. Mechanisms of cardiac arrhythmias. Rev Esp
833 Cardiol (Engl Ed). 2012;65(2):174-85. doi: 10.1016/j.recesp.2011.09.018. PMID: 22192903.

834 81. Trenor B, Romero L, Ferrero JM, Jr., Saiz J, Molto G, Alonso JM. Vulnerability to reentry
835 in a regionally ischemic tissue: a simulation study. Ann Biomed Eng. 2007;35(10):1756-70. doi:
836 10.1007/s10439-007-9353-3. PMID: 17616818.

837 82. Qu Z, Weiss JN. Mechanisms of ventricular arrhythmias: from molecular fluctuations to
838 electrical turbulence. Annu Rev Physiol. 2015;77:29-55. doi:
839 10.1146/annurev-physiol-021014-071622. PMID: 25340965.

840

841 **Supporting information**

842 **S1 Fig.** The variations of electrophysiological characteristics of other ion currents and
843 concentration of single cells in normal, ischemia1a, ischemia1b, and MI conditions.

844 **S2 Fig.** Wave propagation in 2D homogenous tissues: normal, ischemia 1a, ischemia 1b, MI,
845 decoupled 1b and MI. (The time interval of S2 stimulation was 390ms in ischemia 1b condition
846 and the rest was 420ms.)

847 **S3 Fig.** Wave propagation in the 2D tissue where ischemia 1a, decoupled ischemia 1b, and
848 decoupled MI distributed horizontally using the S1-S2 protocol when the S2 stimulus was applied
849 in the (A) lower left or (B) upper left corner.

850 **S4 Fig.** (A) (i) APD distribution in the fifth stimulation in the 2D tissue where ischemia 1a,
851 decoupled 1b, and decoupled MI distributed circularly with a pacing cycle of 420ms. (ii) The
852 maximum APD difference between each cell and its neighbors in the 2D tissue. (B) (i) APD of all
853 cells along the border (line L1) in the 2D tissue. (ii) APD of all cells along the line L2 (with a
854 radius of 350) and L3 (with a radius of 450) in the 2D tissue.

855 **S5 Fig.** Wave propagation in the 3D ventricular tissue (A) with ischemia 1a, (B) with ischemia 1b,
856 or (C) with MI areas (with stimulus interval 310ms and 240ms, respectively).

857 **S6 Fig.** (A) (i) Wave propagation in the 2D tissue where ischemia 1a, decoupled 1b, and

858 decoupled MI distributed horizontally and (ii) VWs when the leftmost stimulation was applied
 859 using the S1-S2 protocol before and after ischemia 1b area was replaced with ischemia 1a area.
 860 **S7 Fig.** (A) Wave propagation in the 2D tissue where ischemia 1a, 1b, and MI distributed
 861 horizontally with gradient distribution of all parameters and (B) action potentials of points P1, P2,
 862 and P3 when the leftmost stimulation was applied with a pacing cycle of 290ms using the dynamic
 863 stimulation protocol.