Mesenchymal Stem Cells Suppress Cardiac Alternans by Activation of PI3K Mediated Nitroso-Redox Pathway

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SUPPLEMENTAL MATERIAL

Short Title: Sattayaprasert, *et al*. Stem Cells Suppress Cardiac Alternans.

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Supplementary Material

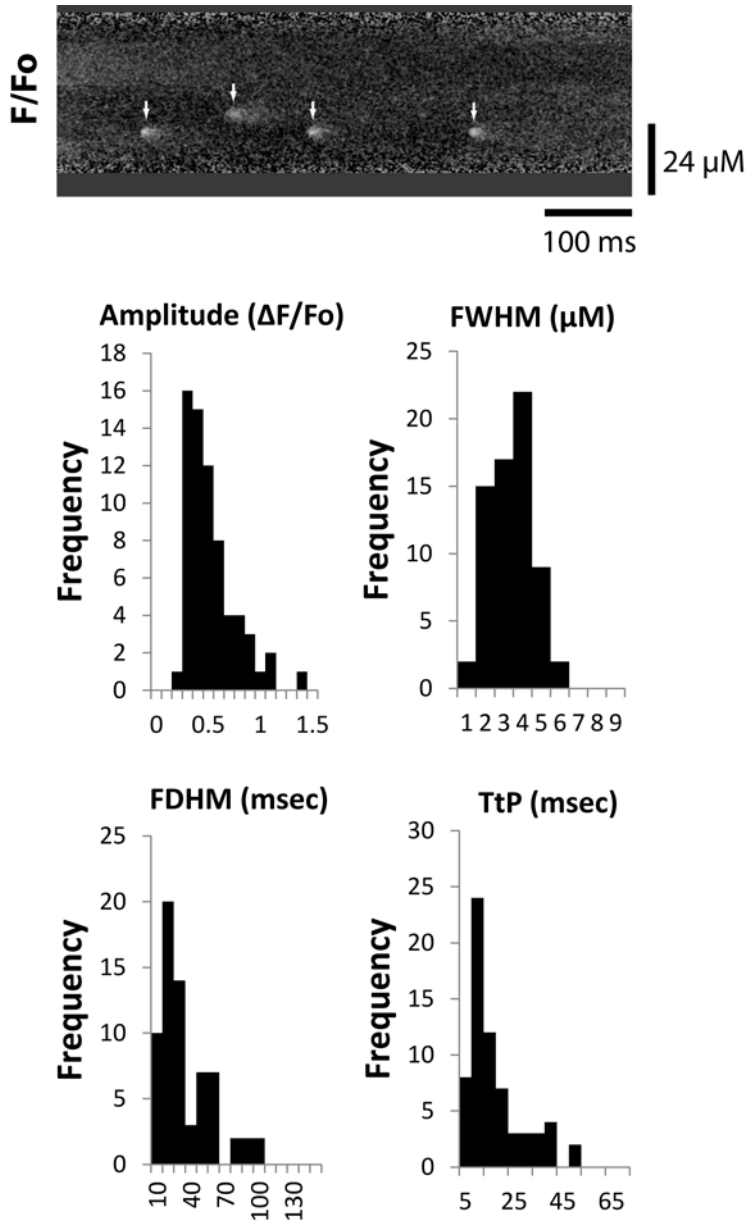
Supplemental Methods

Confocal imaging: Ca^{++} sparks were recorded on day 14-20 from hCM monolayers plated at a density of 50000 cells per 25 mm coverslip. Monolayers were incubated with Tyrodes solution (140 NaCl, 4.56 KCl, 0.73 $MgCl_2$, 10 HEPES, 5.0 dextrose, 1.25 $CaCl_2$) containing 5 μM Fluo-4AM (Sigma/Aldrich) and an equal amount of Pluronic (20%W/V) for 20 minutes. Monolayers were then washed with normal Tyrodes solution before mounting on a bath chamber. Imaging was performed using a Leica TCS SP8 tandem scanner HyD detection confocal mounted to a DMi8 inverted microscope with a 40x 1.3 NA oil immersion objective. The bath chamber was placed in an environmental control incubator attached to a Super Z Galvo stage. Fluo-4AM was excited with 506 nm light from a white light Gen II supercontinuum laser, and emission was collected at >511 nm. The scan line was placed at the middle depth of the cell (Z-axis) and placed to avoid nuclei. Line scan imaging was performed at 512 pixels per line at a scan rate of 1000 lines per second for 8 seconds per recording. All experiments were performed at 28°C. Line scan images obtained during diastole (rest) were analyzed for spark activity using Sparkmaster with recommended settings [2].

Ca^{++} transient imaging: Ca^{++} transients were recorded on day 14-20 from hCM monolayers plated at a density of 50000 cells per 25 mm coverslip. Monolayers were incubated with Tyrodes solution (140 NaCl, 4.56 KCl, 0.73 $MgCl_2$, 10 HEPES, 5.0 dextrose, 1.25 $CaCl_2$) containing 1 μM Fluo-3AM (Sigma/Aldrich) for 20 minutes. Monolayers were then washed with normal Tyrodes solution before mounting on a bath chamber designed for field stimulation. The chamber was mounted on a stage adapter and Fluo-3AM fluorescence (485/530 nm) was measured using an inverted Axiovert fluorescence microscope (Zeiss) with a cooled CCD camera (Princeton Instruments) over a 420 μm by 320 μm field of view. Ca^{++} transients were measured from hCM.

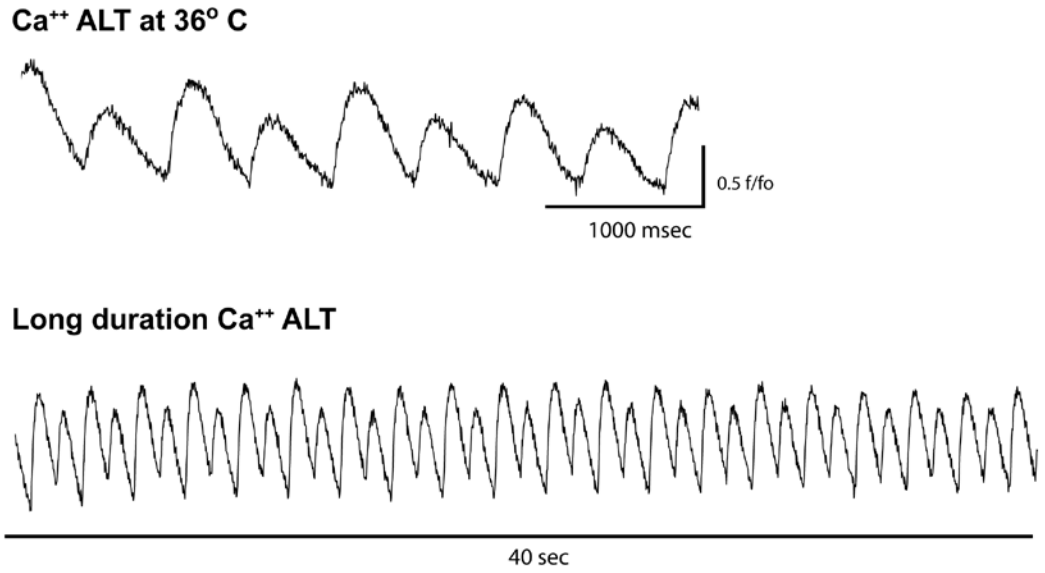
Recordings were performed at room temperature unless indicated otherwise (e.g. Supplemental Figure 2). In some experiments, Isoproterenol (ISO) was then added at 100 nM and pacing and recordings were repeated (Supplemental Figure 3).

Supplemental Figure 1



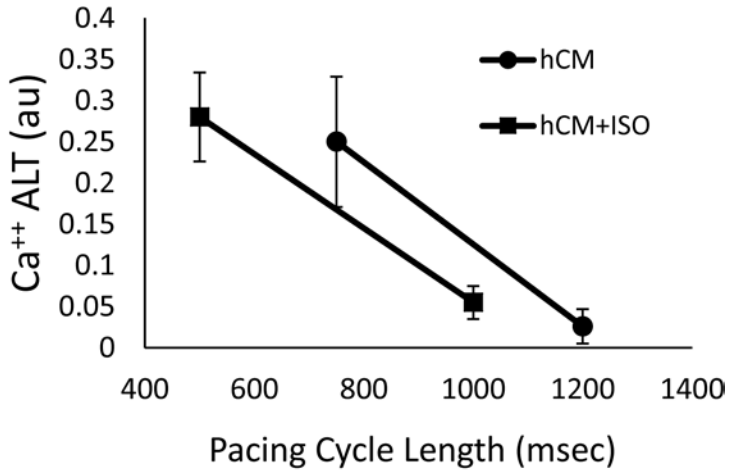
Supplemental Figure 1: Ca^{++} spark activity measured in hCM monolayers during spontaneous activity. Top shows a single line scan image of F/Fo measured during diastole from a single hCM that exhibited 4 individual Ca^{++} sparks (arrows). Similar line scan images were obtained from a total of 15 hCM in 4 monolayers. Sparks were analyzed to determine amplitude (F/Fo), full width at half maximum amplitude (FWHM, µM), full duration at half maximum amplitude (FDHM, msec), and time to peak (TtP, msec).

Supplementary Figure 2



Supplemental Figure 2: Example of Ca^{++} alternans (ALT) measured from hCM monolayer with H_2O_2 maintained at 36ºC (top) while pacing at 500 msec cycle length. At 36ºC, the pacing cycle length at which alternans occurs is lower than that at colder temperatures, as shown previously [1]. Middle trace shows steady state Ca^{++} ALT with H_2O_2 measured over 40 sec while pacing at 750 msec cycle length. Bottom trace shows the pacing cycle length dependence of Ca^{++} ALT in hCM monolayer treated with H_2O_2 at room temperature. These results demonstrate that Ca^{++} ALT occurs at body temperature, is stable over long durations, and exhibits rate dependence.

Supplemental Figure 3



Supplemental Figure 3: Effect of Isoproterenol on Ca^{++}alternans (ALT) in hCM monolayers without MSCs and H_2O_2. Shown are Ca^{++} ALT measured in hCM monolayers with Isoproterenol (ISO, 100 nM) while pacing at the cycle lengths indicated. ISO reduced Ca^{++} ALT as indicated by a left shift of its pacing cycle length dependence.

Supplementary References

[1] Hirayama Y, Saitoh H, Atarashi H, Hayakawa H. Electrical and mechanical alternans in canine myocardium in vivo: Dependence on intracellular calcium cycling. Circulation. 1993;88:2894-902.

[2] Picht E, Zima AV, Blatter LA, Bers DM. SparkMaster: automated calcium spark analysis with ImageJ. Am J Physiol Cell Physiol. 2007;293:C1073-81.