Kinetics parameters, TTP and RT_{50}, were significantly increased in nucleoplasmic and cytoplasmic compartments (TTP: 254.8 ± 16.9ms and 60.9 ± 8.9ms vs 366.9 ± 19.7ms and 123 ± 11.6ms; RT_{50}: 651.6 ± 42.7ms and 570.5 ± 29.3ms vs 758.2 ± 28.1ms and 657.1 ± 42.8ms, $P<0.05$), suggesting the slower spread of CaTs throughout the cells isolated from end-stage failing myocardium.
These results show that there are significant alterations of kinetic parameters and amplitudes of nucleoplasmic and cytoplasmic [Ca] transients in condition of end-stage heart failure.

517-Pos Board B303
Structured Culture Scaffolds Improve the Calcium Handling Properties of Cardiomyocytes Differentiated from Induced Pluripotent Stem Cells
Christopher Rao, Themistoklis Prodromakis, Umar Chaudhry, Patrizia Camelliti, Magdi H. Yacoub, Ara Darzi, Nadire N. Ali, Thanos Athanasiou, Cesare M. Terracciano.
Imperial College London, London, United Kingdom.
Induced Pluripotent Stem Cells derived Cardiomyocytes (iPSC-CM) have been proposed as in vitro models of myocardial physiology and disease; however a significant obstacle is their immature phenotype which is poorly representative of adult myocardium. We hypothesise that culture scaffolds which force the alignment of iPSC-CM will improve important determinants of cellular function such as Ca2+ handling properties.
iPSC-CM (Cellular Dynamics, Wisconsin) were seeded onto fibronectin coated microgrooved polydimethylsiloxane (PDMS) scaffolds (MS) fabricated using photolithography, or on unstructured PDMS membrane (UM). After two weeks in culture iPSC-CM were loaded with Fluo-4 AM. Confocal microscopy was used in line scanning mode whilst iPSC-CM were beating spontaneously, at 1Hz and 0.5Hz.
iPSC-CM cultured on MS had a shorter time to peak Ca2+ transient amplitude (tP) when stimulated at 1Hz (Mean+/-SEM: MS: 145+/-6ms, n=37; UM: 200+/-10ms, n=58; p=0.0002, Mann Whitney Test) and time to 50% transient decay (t50) (Mean+/-SEM: MS: 222+/-8ms, n=37; UM: 258+/-8ms, n=58; p=0.0065) but no change in time to 90% transient decay (t90). At 0.5Hz there was a shorter tP (Mean+/-SEM: MS: 199+/-11ms, n=37; UM: 242+/-10ms, n=64; p=0.0073) but no changes in t50 or t90. Similarly while iPSC-CM were beating spontaneously, there was a reduced tP (Mean+/-SEM: MS: 223+/-19ms, n=18; UM: 280+/-11ms, n=37; p=0.00121). The spontaneous beating rate was unchanged between groups. SR Ca2+ release was elicited using 50mM caffeine and could be obtained consistently in 80% (n=38) of MS but only 10% (n=17) of UM suggesting that SR Ca2+ regulation is improved by culture on MS.
We conclude that structured culture improves Ca2+ cycling in iPSC-CM to make them more representative of adult human myocardium, our provisional data suggests this is related to sarcoplasmic reticulum maturity however further characterization is required.

518-Pos Board B304
Synchronization of Ca+2 Release in Multicellular Cardiac Preparations
Jessica L. Slabaugh, Lucia Brunello, Sandor Gyorke, Paul M.L. Janssen.
Ohio State University, Columbus, OH, USA.
Delayed afterdepolarizations (DADs) are frequently observed under conditions that increase cellular and SR Ca+2. The high Ca+2 can cause spontaneous SR Ca+2 release and oscillations in the membrane potential. It is these oscillations that evoke spontaneous action potentials in the myocytes, leading to arrhythmias. Since myocytes are electrically coupled, any depolarization that arises in one myocyte would be dampened due to dissipation of the current in the neighboring myocytes. It is believed that some synchronization mechanism exists that synchronizes DADs and action potentials in neighboring cells. In our current study, we are interested in examining whether there is a synchronization mechanism for the generation of triggered activity in multicellular cardiac preparations. Trabeculae isolated from rat hearts were mounted on a modified confocal microscope that was equipped with a force transducer. Trabeculae were loaded with Rhod-2 AM, and were washed with buffer containing BDM and blebbistatin to stop contraction and allow for Ca+2 imaging. Combination of 0.5 mM caffeine and a range of concentrations of isoproterenol (100–300 nM) were added to the perfusion to induce DADs. Line scans were acquired and fluorescence emitted were expressed as ΔF/F0. The addition of caffeine and isoproterenol show an increased incidence of DADs, with more DADs occuring as the concentration of isoproternol was increased. The amplitude of the DADs, in comparison to the stimulated twitch, was also increased with increasing isoproternol concentration. In similar experiments without BDM/blebbistatin, examining the force tracings, the DADs occured in a synchronous manner in neighboring myocytes eventually leading to contractions that are similar to those that were paced. The line scan images also show similar findings, where the Ca+2 transient amplitudes were analyzed. Collectively, these results suggest that intracellular, diastolic SR Ca release is can become synchronized in a multicellular cardiac preparation.

519-Pos Board B305
Synchronization of Local Calcium Releases by Beta-Adrenergic Stimulation in Cardiac Pacemaker Cells
Oliver Monfredi[1,2], Edward G. Lakatta[2], Victor A. Maltsev[2].
[1]University of Manchester, Manchester, United Kingdom,
[2]NIA/NIH, Baltimore, MD, USA.
Sympathetic heart rate acceleration is a complex orchestration of intercellular, subcellular and molecular events. According to a modern concepts, rhythmic local Ca^{2+}releases (LCRs), generated by a subcellular ''Ca-clock'', interact with a ''membrane-voltage-clock'', forming a coupled-clock system that produces rhythmic action potentials (AP). We imaged LCRs using a fast-2D-camera with simultaneous AP recording via perforated-patch-clamping to explore how β-AR stimulation modulates the phase and synchronization of the ensemble of individual LCRs within a given diastole to accelerate spontaneous beating rate. In single rabbit SA-node cells (36°C) isoproterenol (100nM): (i) synchronized LCR occurrence [the standard deviation of individual LCR periods (time from Ca^{2+}transient peak to LCR occurrence) decreased (−30%; baseline,71.5ms vs. ISO,50ms)], (ii) shifted the LCR phases to earlier time-point, (iii) shifted ''Ca-nadir'', i.e. time-point when AP-induced Ca^{2+}-transient decay ceases and $[Ca^{2+}]$ begins to increase due to the occurrence of LCRs (time from maximum-diastolic-potential to Ca-nadir: baseline, 103ms vs. ISO,86ms); (iv) accelerated diastolic depolarization (baseline,0.09mV/ms vs. ISO,0.15mV/ms); (v) shortened spontaneous cycle length (baseline,420ms vs. ISO,345ms). We previously had demonstrated a close relationship between the decay of the global AP-induced Ca^{2+} transient and the LCR phase (within a given cycle) and the AP cycle length; we now found that the decays of each LOCAL Ca^{2+}Transient (LCaT) induced by the prior AP vary in different subcellular locations/neighborhoods and that isoproterenol not only accelerated LCaT-decays (average time constant τ: baseline, 104 vs. ISO,87ms) but also synchronized LCaT-decays among neighborhoods (τ's SD/mean: baseline,40.5% vs. ISO,33.7%). More synchronous LCaT-decay mirrors more synchronous local SR Ca^{2+}-pumping and therefore more synchronous achievement of the threshold SR Ca load required for spontaneous LCR occurrence. Thus, earlier and more synchronized LCR occurrence under β-AR stimulation insures an earlier and larger Na^{+}/Ca^{2+}-exchanger current that ultimately accelerates diastolic depolarization rate.

520-Pos Board B306
Effects of Cytosolic Ca Buffering on Sarcoplasmic Reticulum Ca Leak in Permeabilized Rabbit Ventricular Myocytes
Elisa Bovo[1], Stefan R. Mazurek[1], Michel Fill[2], **Aleksey V. Zima**[1].
[1]Loyola University Chicago, Maywood, IL, USA, [2]Rush University Medical Center, Chicago, IL, USA.
In this study we tested the hypothesis that local Ca-induced Ca release (CICR) within ryanodine receptor (RyR2) release clusters plays an important role in defining spark-mediated sarcoplasmic reticulum (SR) Ca leak. We studied to what degree changes of cytosolic Ca buffer capacity affect single RyR activity in lipid bilayers, Ca spark properties, and SR Ca leak in permeabilized ventricular myocytes. No substantial changes in single RyR2 function were observed if BAPTA (a fast Ca buffer) was added (holding free Ca constant) to the EGTA-buffered cytosolic solution. However, the same cytosolic BAPTA addition almost completely eliminated Ca sparks in cells. The elimination of Ca sparks by BAPTA was not due to a decrease in SR Ca load. The addition of the fast cytosolic Ca buffer significantly decreased SR Ca leak, particularly at high $[Ca]_{SR}$ (>400 μM) where Ca sparks significantly contribute to SR Ca leak. In contrast, lowering cytosolic Ca buffer capacity (by decreasing EGTA from 0.36 to 0.1 mM; keeping free Ca constant) drastically increased Ca spark frequency and width, leading to generation of multifocal (or propagating) Ca sparks. This decrease in cytosolic Ca buffer capacity also significantly accelerated SR Ca leak as a result of augmentation of Ca spark-mediated component of SR Ca leak. In conclusion, by changing cytosolic Ca buffering (capacity or affinity) we were able to experimentally separate Ca spark- and non-spark- mediated component of the overall SR Ca leak in ventricular myocytes. These data also suggest that changes in the dynamics of local cytosolic Ca handling (buffering, extrusion, etc.) are physiologically important in determining the degree of spark and non-spark mediated SR Ca leak.