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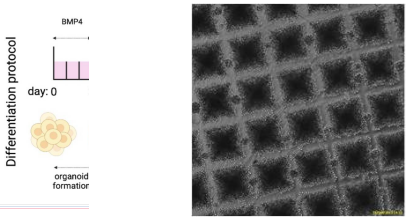
ELECTROMECHANICAL CELL-BASED BIOSENSORS FOR DRUG AND DISEASE OF 3D CARDIAC MODELS

Martin Pešl

Stem cell derived cardiomyocytes (iPSC-CMs)

- cardiac organoid model (embryonic body EB)



Pesl et al. Heart and Vessels 2014

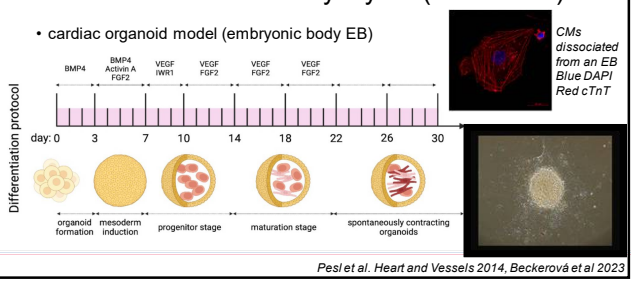
Arrhythmia and Heart Failure preclinical management

- screening responses of new developed compounds
- improvement of drug and treatment strategies

- *in silico*
- *in vitro*
- small animal models
- large animal models
- healthy and diseased human participants

Stem cell derived cardiomyocytes (iPSC-CMs)

- cardiac organoid model (embryonic body EB)



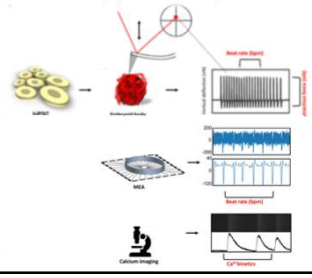
CMs dissociated from an EB Blue DAPI Red cTnT

Pesl et al. Heart and Vessels 2014, Beckerová et al 2023

Cardiac risk assessment

- *in vitro* Electrophysiology, **no standardized contractlity safety studies**
  - I_{Kr} assays (adequate to QT assays) ICH S7B guidance EMA / similar FDA
  - *multi-ion channel* comprehensive proarrhythmia assay (CiPA, *Fermini 2016*)
- Animal Cardiomyoctes
  - zebrafish with two heart chambers, rodents with aberrant ion channel levels
  - dog, porcine and ovine cells with subtle differences and very complex handling
- Stem cell derived cardiomyocytes (iPSC-CMs)
  - spontaneous beat and partially immature phenotype
  - single cells lack organ-dependent factors and cell-cell interactions
  - cardiac organoid model approximates an optimal translational value and repeatability
    - enables both safety and disease phenotype studies

Phenotype analysis of iPSC-CMs

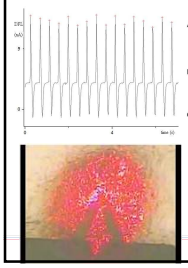


Atomic force microscopy
- cantilever deflection reflects contraction/relaxation

Microelectrode array
- field potential reflects syncytium action potential

Calcium Imaging
- intrinsic Ca^{2+} kinetics using calcium-binding Fluo-8

Atomic force microscopy of iPSC-CMs



Contraction
- first cantilever deflection in „QRS" complex

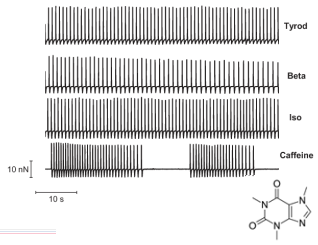
Iso line in between the beats
- cantilever excercise 3-6nN force on the surface of cluster

Relaxation
- due to constant force cantilever follow (down) relaxing EB without loosing the contact

contraction rate, relaxation time, displacement / deflection - computed contraction force

Pesl et al. Heart and Vessels 2014

Atomic force microscopy of iPSC-CMs



Tyrod solution, Ca^{2+} 1,8mmol

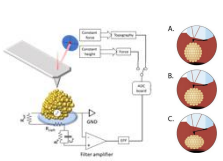
Beta blocking Metoprolol

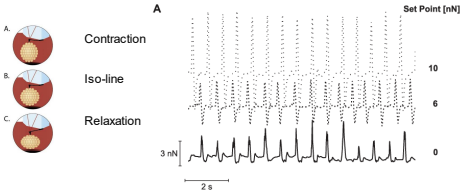
Beta stimulation Isoproterenol / Adrenalin

Calcium release methylxanthie - Caffein

Klimovic, Scurek, Pesl, Front Pharmacol. 2022

Atomic force microscopy of iPSC-CMs





Contraction

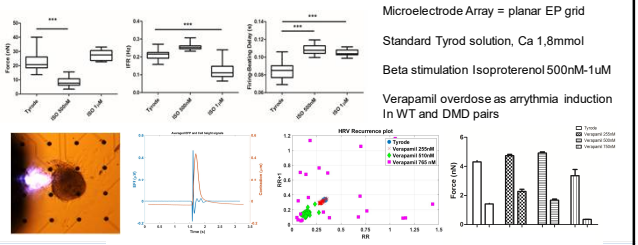
Iso-line

Relaxation

Set point = mimicking „afterloading" resitance, preload mimicked by cantilever elasticity (drag)

Pesl et al. Biosens. Bioelectron 2016

Combined AFM and MEA on iPSC-CMs



Microelectrode Array = planar EP grid

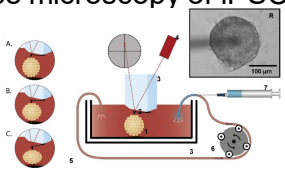
Standard Tyrod solution, Ca 1,8mmol

Beta stimulation Isoproterenol 500nM-1uM

Verapamil overdose as arrythmia induction In WT and DMD pairs

Caluori, Biosens. Bioelectron 2019

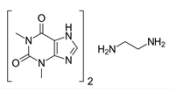
Atomic force microscopy of iPSC-CMs



contraction rate, relaxation time and force with influence of selected drugs and in mutated cell lines
Tyrod´s solution - stabilization- Drug administration - stabilization- Wash out - stabilization- Wrap up

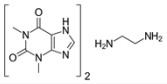
Klimovic, Scurek, Pesl, Front Pharmacol. 2022

AFM drug testing



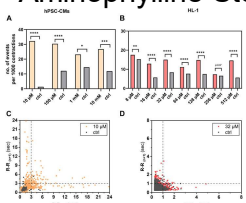
Klimovic Scurek Pesl Front Pharmacol.2022

AFM drug testing

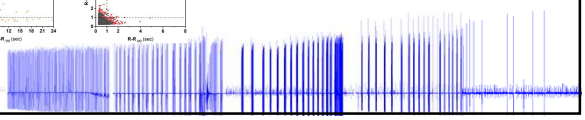


Klimovic Scurek Pesl Front Pharmacol.2022

Aminophylline Stop&go effect



- different cutoff R-R values tested
- assessed the number of R-R intervals longer than 3s or 1s, respectively.
- higher occurence of „stops“ than in non-treated controls in both cut-off models



Klimovic Scurek Pesl Front Pharmacol.2022

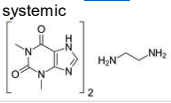
AFM drug testing of Aminophilline

Theophylline with Ethylenediamine 2:1

nonselective adenosine receptor antagonist
competitive nonselective phosphodiesterase inhibitor

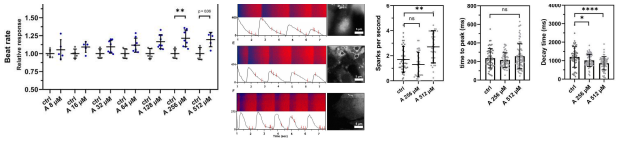
bronchodilation adjunct to inhaled beta-2 selective agonists and systemic corticosteroids

clinically relevant concentrations 20-110 μmol/l



Klimovic Scurek Pesl Front Pharmacol.2022

Aminophylline Stop&go effect

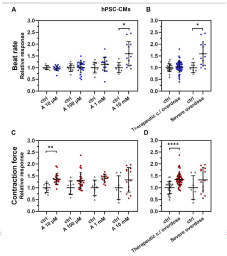


- positive chronotropy with increasing concentration of aminophylline
- linear contraction rate increase in the cells treated with 256 μM aminophylline, similar non-significant trend at 512-μM treatment

- intracellular cytosolic Ca^{2+} in HL-1 cells
- higher concentrations of aminophylline calcium leakage (sparks), shorter decay time = arrhythmic events

Klimovic Scurek Pesl Front Pharmacol.2022

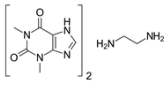
AFM drug testing of Aminophylline



- linear concentration-dependent positive chronotropic effect of aminophylline significant up to the 10 mM (severe overdose)
- Increased varition in beat to beat analysis (SDNN) = arrythmia
- Mild, but statistically significant increase inotropy (contraction force of EBs) already in lower concentrations of aminophylline
- washout decrease effects during the washout period = suggesting that the chronotropic and inotropic effects is likely due to the effect of aminophylline and not due to irreversible cellular damage

Klimovic Scurek Pesl Front Pharmacol.2022

Aminophylline dual Arrythmogenic effect



- concentration-dependent (“deterministic”) effect, presenting with an increased beat rate (potential clinical correlate: sinus tachycardia),
- concentration-independent (“stochastic”) effect, characterized by tachycardia-like episodes alternating with long pauses (potential clinical correlate: atrial fibrillation).

New parameters for cardiotoxicity vs. safety testing of various molecules or drugs.

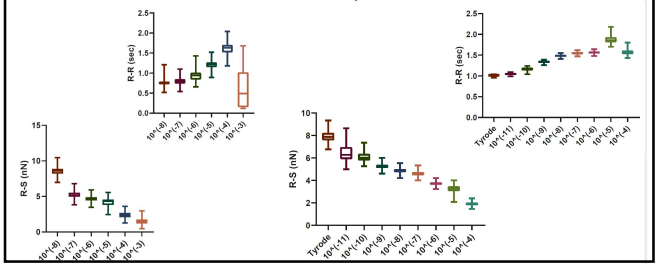
Klimovic Scurek Pesl Front Pharmacol.2022

Salbutamol

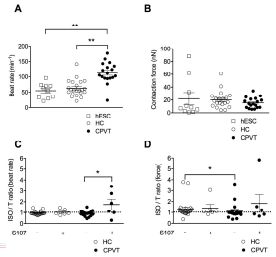
- Agonist of β_2 receptors
- **10^{-8} up to 10^{-3}M concentration**

Ipratropium

- Antagonist of muscarinic acetylcholine receptors
- **10^{-11} up to 10^{-4}M concentration**



AFM CPVT-iPSC-CMs



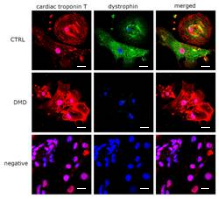
Resting beat rate and contraction force for hES WT, Healthy Control HC and CPVT-EBs at rest.

CPVT-EBs have a weaker and/or abnormal rhytm response to ISO in force and beat rate.

Balanced by Ca stabilizer Rycals (S107)
- positive chronotropic effect on CPVT-EBs when compared to untreated CPVT-EBs / no effect on HC-EBs

Acimovic et al. 2018

AFM disease harbouring iPSC-CMs



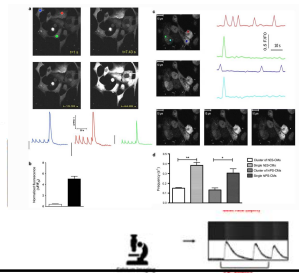
Disease modelling from patient biopsies

- neuromuscular diseases
- channelopathies

Duchenne muscular dystrophy DMD

Catecholaminergic polymorfic ventricular tachycardia CPVT

Calcium Imaging of iPSC-CMs



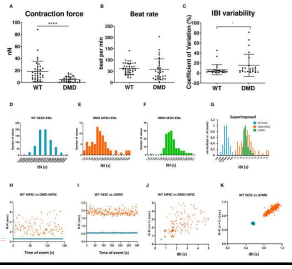
Ca^{2+} imaging showing cytosolic Ca^{2+}
- Spontaneous variations in fluorescence intensity in some cells with the time (t) in seconds. normalized to the basal level
- Induced cytosolic Ca^{2+} elevations upon 10 mM caffeine

b the normalized fluorescence intensity related to spontaneous Ca^{2+}ts (*white bar*, $n = 11$) and caffeine-induced Ca^{2+}ts (*black bar*, $n = 11$).

d the frequency (per second) of spontaneous Ca^{2+} transients in
dissociated clusters of hESC-CMs (*white bar*, $n = 10$),
single dissociated hESC-CMs (*white and striped bar*,$n = 3$),
dissociated clusters of hiPSC-CMs (*gray bar*, $n = 7$)
single dissociated hESC-CMs (*gray and striped bar*, $n = 6$).
Data are presented as mean ± SEM. $^*P < 0.05$, $^{**}P < 0.0$

Pesl et al. 2014

AFM DMD-iPSC-CMs



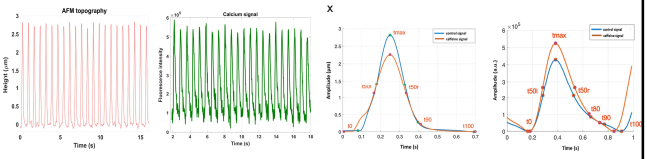
EBs lacking dystrophin showed weaker contraction force and signs of arrhythmias (beat rate not affected).
($n = 36$ for WT, $n = 29$ DMD / $n = 38$ for WT, $n = 31$ DMD)

Superimposed representative inter-beat-interval (IBI)
- different variability in recorded IBI but also prevalence in shorter IBI in WT-EBs compared to DMD-EBs

Poincaré plots of control hiPSC with DMD-hiPSC-EBs ($n = 145$ time points for each EB)
- more scattered inter-contraction pattern in DMD-EBs compared to WT-EBs

Jelinkova et al. 2020

AFM and Calcium Imaging of iPSC-CMs



Simultaneous Ca^{2+} cycling and contractility of CM organid

The increase peak amplitude and elongated width of Ca^{2+} transients can also be explained with the stimulated and prolonged depletion of the SR storage by Caffein.

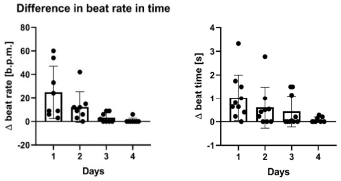
Caluori et al. 2019

Rhythm diseases

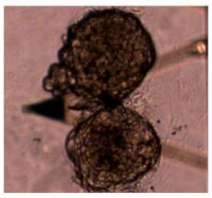
Focal activation
- single cell model
- animal models
- multicellular clusters

Propagation
- cannot be studied on single cells
- partially relevant in animal models,
- limited in small cell clusters

Synchronizing two clusters



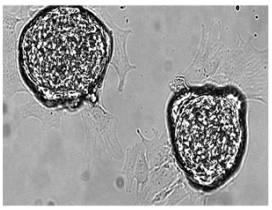
synchronized twins



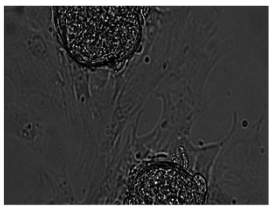
ImageJ/Fiji macro Musclemotion

Pivato et al. 2022

Combining two clusters

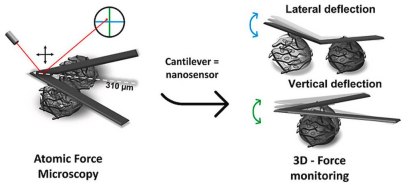


synchronized twins



Pivato et al. 2022

AFM monitoring two clusters

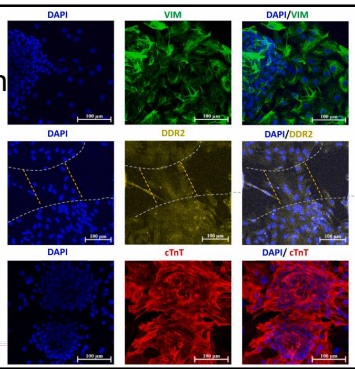


JPK/Bruker Nanowizard 3
MLCT-C cantilever Bruker
- partially coated silicon nitride cantilever

force spectroscopy mode
1 nN setpoint (active mode)
- allows tracing of cantilever/ cluster drift and loss of contact
- mechanical response natural to cardiac cells - as preload and afterload

Pivato et al. 2022

Combin



seeding clusters in agarose mask

200um apart

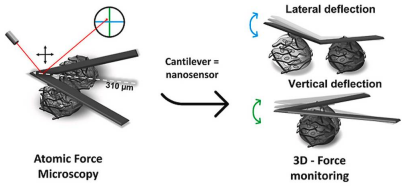
within 96h after seeding forming conductive „bridge“

synchronizing contraction pattern

DDR2 Discoidin Domain Receptor
cTnT Cardiac Troponin T
VIM Vimentin

Pivato et al. 2022

AFM monitoring two clusters



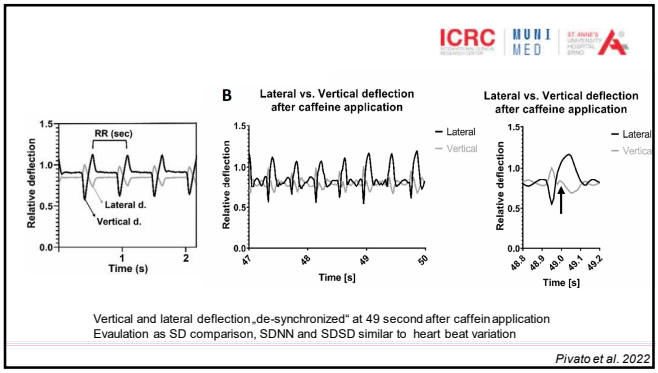
vertical and lateral deflection mechanocardiograms (MCG) collected in 100 Hz sampling frequency in a pilot group

vertical deflection = contraction frequency (R-R interval)

lateral (horizontal) deflection remains stable (repeating curve) until desynchronization

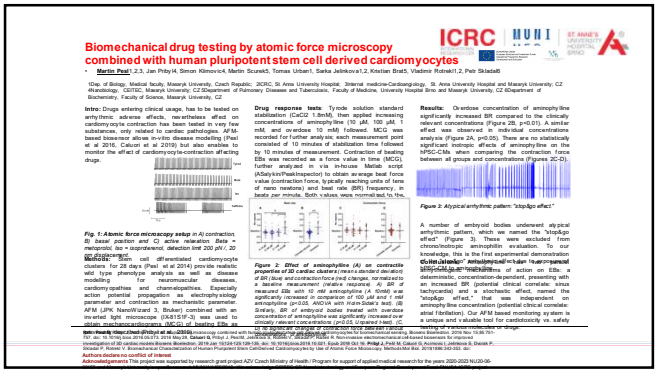
Data extraction performed by in-house made python script

Pivato et al. 2022



Vertical and lateral deflection „de-synchronized" at 49 second after caffein application
Evaulation as SD comparison, SDNN and SDSD similar to heart beat variation

Pivato et al. 2022



Biomechanical drug testing by atomic force microscopy combined with human pluripotent stem cell derived cardiomyocytes

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Intro: Drugs entering clinical useage, has to be tested on arrhythmic adverse effects, nevertheless effect on cardiomyocyte contraction has been tested in very few substances, only related to cardiac pathologies. AFM-based biosensor allows in-vitro disease modelling (Pesl et al 2016, Caluori et al 2019) but also enables to monitor the effect of cardiomyocyte-contraction affecting drugs.

Drug response tests: Tyrode solution standard stabilization (CaCl2 1.8mM), then applied increasing concentrations of aminophylline (10 μM, 100 μM, 1 mM, and overdose 10 mM) followed. MCG was recorded for further analysis; each measurement point consisted of 10 minutes of stabilization time followed by 10 minutes of measurement. Contraction of beating EBs was recorded as a force value in time (MCG). Further analysed in in-house Matlab script (ASeq/xlm/Peak/inspector) to obtain average beat force value (contraction force, typically reaching units of tens of nano newtons) and beat rate (BR) frequency, in beats per minute. Both values were normalised to the,

Results: Overdose concentration of aminophylline significantly increased BR compared to the clinically relevant concentrations (Figure 2B, p<0.01). A similar effect was observed in individual concentrations analysis (Figure 2A, p<0.05). There are no statistically significant inotropic effects of aminophylline on the hPSC-CMs when comparing the contraction force between all groups and concentrations (Figures 2C-D).

Figure 3: Atypical arrhythmic pattern: "stop&go effect"

A number of embryoid bodies underwent atypical arrhythmic pattern, which we named the "stop&go effect" (Figure 3). These were excluded from chronotropic aminophilin evaluation. To our knowledge, this is the first experimental demonstration of this unusual arrhythmic event in human cardiac tissue.

Conclusion: Aminophylline's mechanism of action on EBs: a deterministic, concentration-dependent, presenting with an increased BR (potential clinical correlate: sinus tachycardia) and a stochastic effect, named the "stop&go effect," that was independent on aminophylline concentration (potential clinical correlate: atrial fibrillation). Our AFM based monitoring system is a unique and valuable tool for cardiotoxicity vs. safety testing of pharmacological or other

Fig. 1: Atomic force microscopy setup in A) contraction B) basal position and C) active relaxation. Beta = metoprolol, Iso = isoprotenerol, detection limit 200 pN/, 20 beats per minute.

Figure 2: Effect of aminophylline (A) on contractile properties of 3D cardiac clusters (mean± standard deviation) of BR (blue) and contraction force (red) changes, normalized to a baseline measurement (relative response). A) BR of measured EBs with 10 mM aminophylline (A 60/60) was significantly increased in comparison of 100 μM and 1 mM aminophylline (p<0.05, ANOVA with Holm-Sidak's test). (B) Force of contraction was not significantly changed by concentration of aminophylline and, instead, more-over clinically relevant concentrations (p<0.05, Unpaired t-test). (C-

Pesl M, Pribyl J, Klimovic S, Scurek M, Urban T, Jelinkova S, Brat K, Rotrekl V, Skladal P. Biomechanical Characterization of Human Pluripotent Stem Cell-Derived Cardiomyocytes by Use of Atomic Force Microscopy. Methods Mol Biol. 2019;1896:343-353. doi:

Authors declare no conflict of interest
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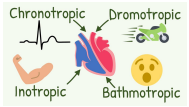
AFM CMs monitoring

Standalone and combined method for topography, rhytmicity and contractility.

Quantifing cardiac chronotrophy, inotrophy, also dromotrophy (Dual clusters) and bathmotrophy (Calcium imaging).



Clear differences in disease models, for testing new therapeutic strategies

Robust model for advanced drug screening and disease-drug interaction.

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