Dual cardiomyocyte cluster arrhytmias detected by atomic force microscopy

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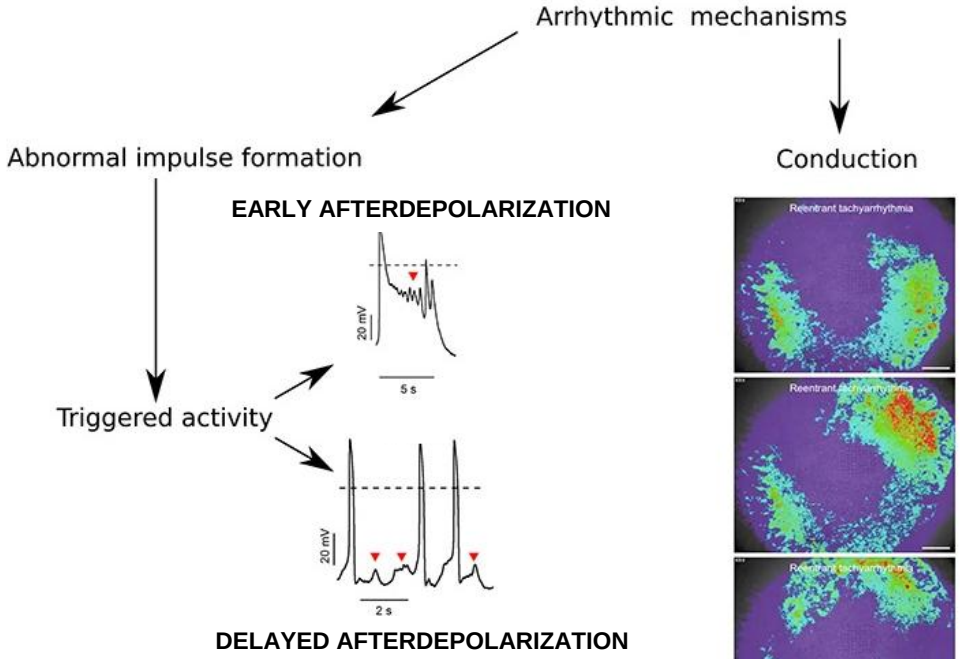
Department of Biochemistry, Faculty of Science, Masaryk University, Brno, Czech Republic
Central European Institute for Technology, Masaryk University, Brno, Czech Republic

Rhythm diseases



Conduction defects

<u>Impulse Propagation</u>

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

<u>Abnormal impulse formation</u>

- can be studied on single cells or single channel (patch clamp)

Adapted from *Paci, J.Cardiovasc.Pharmacol. 2020*

Stem cell derived cardiac organoid/beating syncitium

Functional cardiomyocyte in the dish…

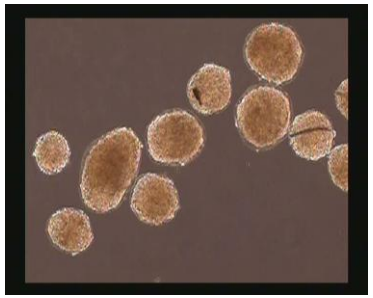
Patient biopsy

Healthy control

reprogramming

disease hiPSC

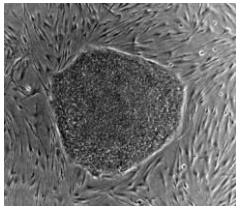
WT hiPSC



CARDIAC ORGANIOD

✓ **SPONTANEOUSLY BEATING**

✓ carrying patient mutation

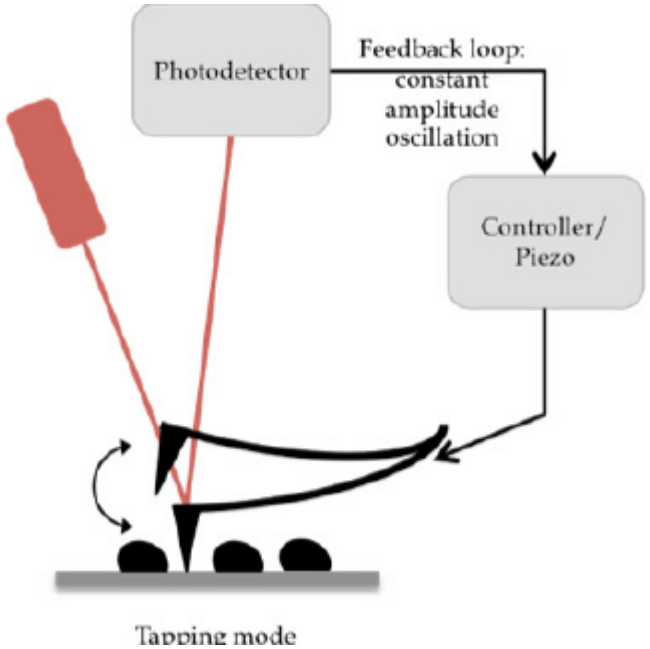


Human Embryonic Stem Cell

WT hESC

Heart and Vesses 2014
Stem Cell Res, 2019

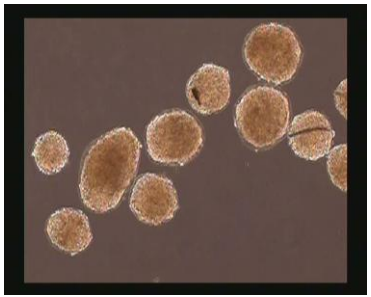
Stem cell derived cardiac organoid/beating syncitium



Atomic Force Microscope (AFM)

Kyeyune (2017). Atomic Force Microscopy.

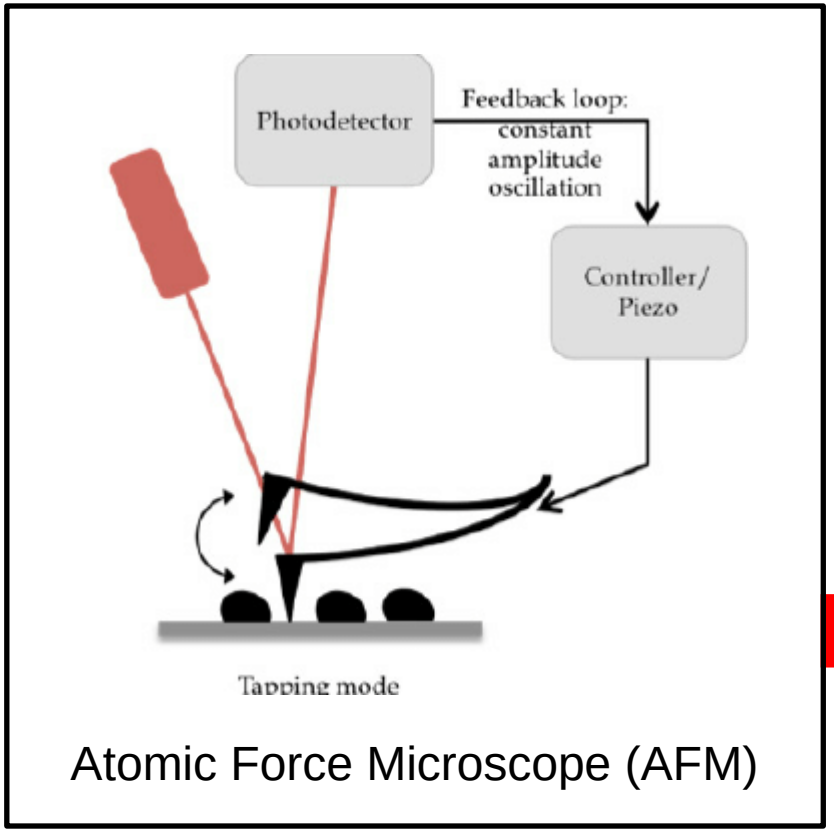
Functional cardiomyocyte in the dish…



CARDIAC ORGANIOD

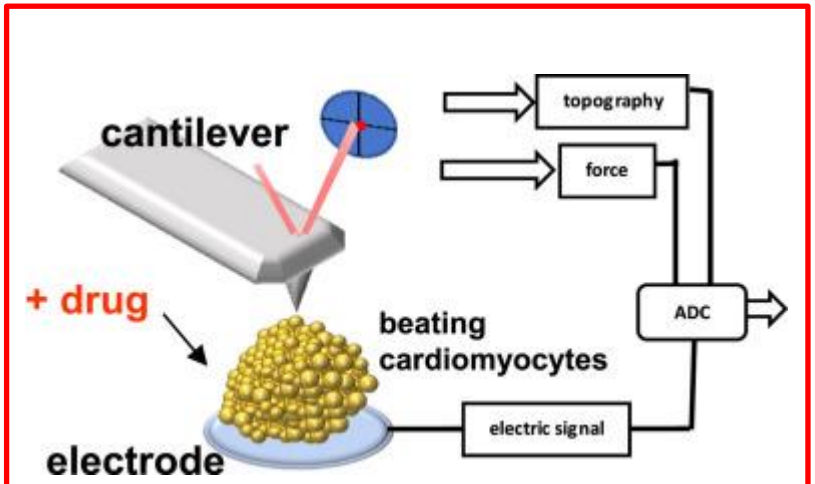
✓ **SPONTANEOUSLY BEATING**

Cardiac organoid biosensor



Atomic Force Microscope (AFM)

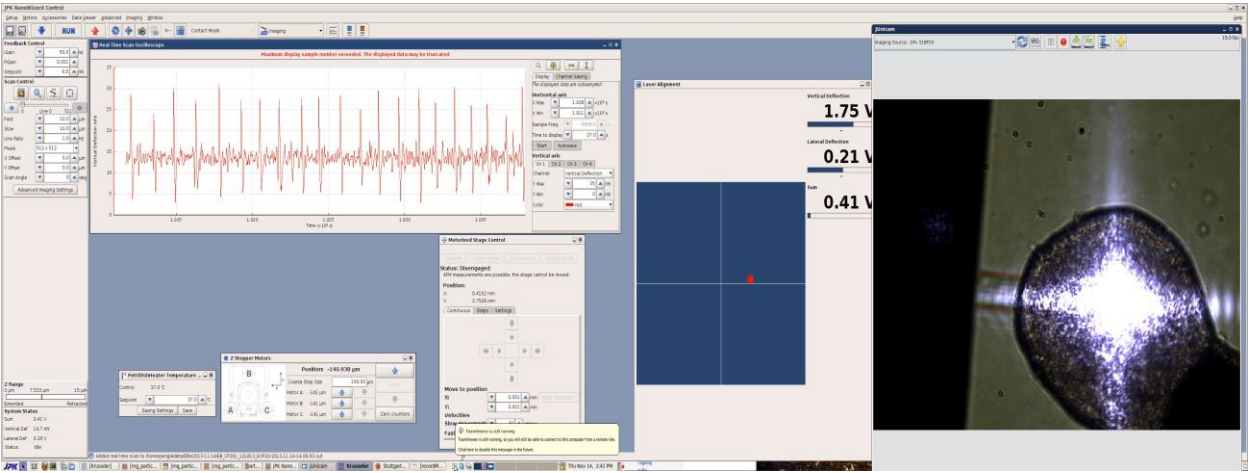
AFM coupeled with cardiac organoid



Real time force and frequency of contraction monitoring
-allows for drug effect monitoring

Heart and Vesses 2014
Stem Cell Res, 2019

Real time drug arrhytmogenicity monitoring

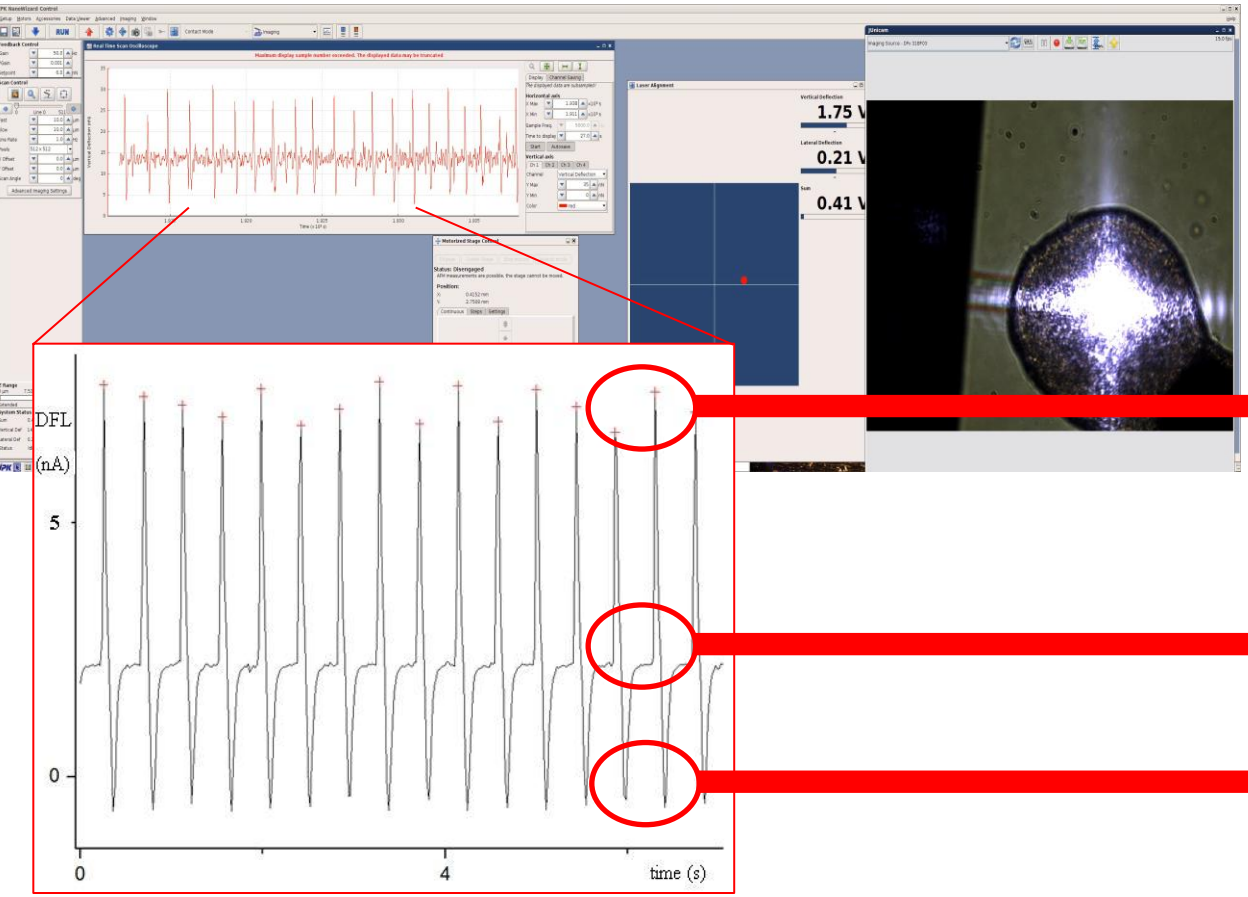


AFM-based biosensor setup
stem cell derived CMs cluster

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

Biosens. Bioelectron 2016

Real time drug arrhytmogenicity monitoring



AFM-based biosensor setup
stem cell derived CMs cluster

Force [nN]:

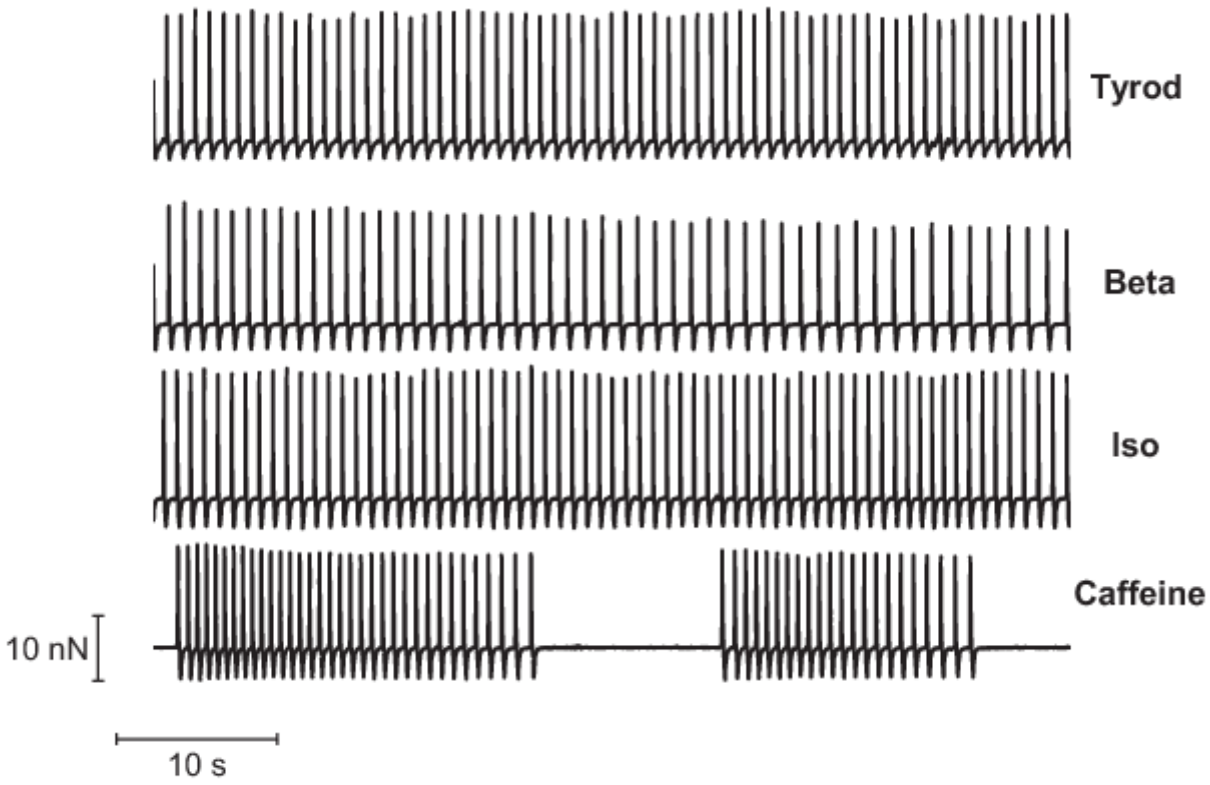
A. Contraction

B. Relaxation

C. Displacement

Biosens. Bioelectron 2016

Real time drug arrhytmogenicity monitoring

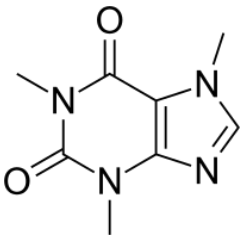


standard pharmacological indicators of different phenotypic features

beta adrenergic blocking
Metoprolol

beta adrenergic stimulation
Isoproterenol / Adrenalin

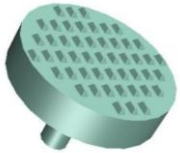
Methylxanthie
Caffeine



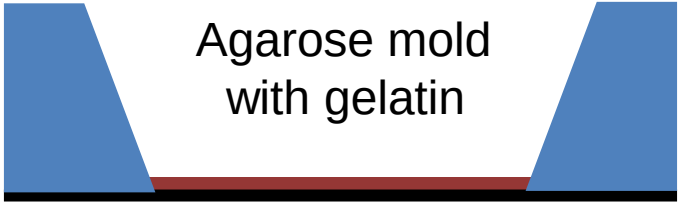
But this does not monitor conductive defects!

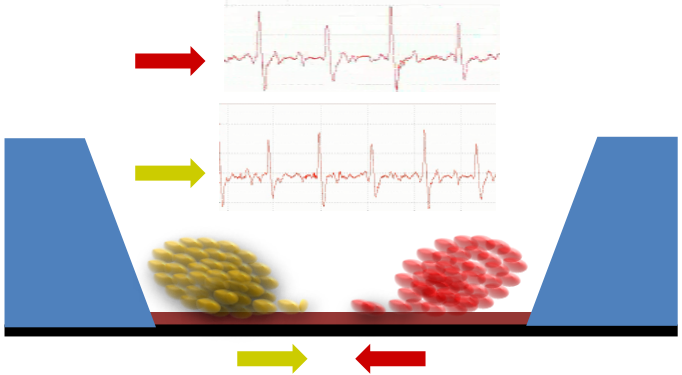
Klimovic et al. Front Pharmacol.2022
AFM Biomed Munster, 2019

Real time drug arrhytmogenicity monitoring – conductive defects



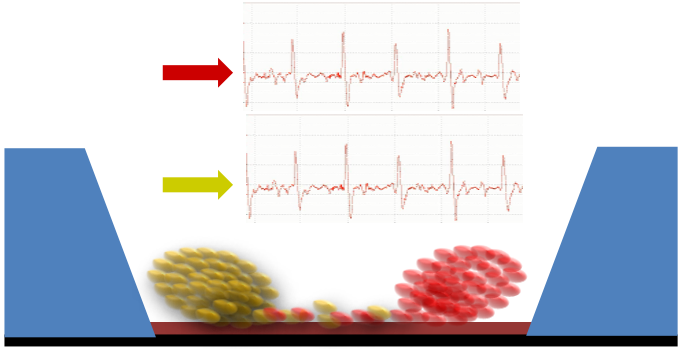
3D printed stamp





Seeded 2 asynchronous organoids

Overgrowth of cardiac myofibroblasts



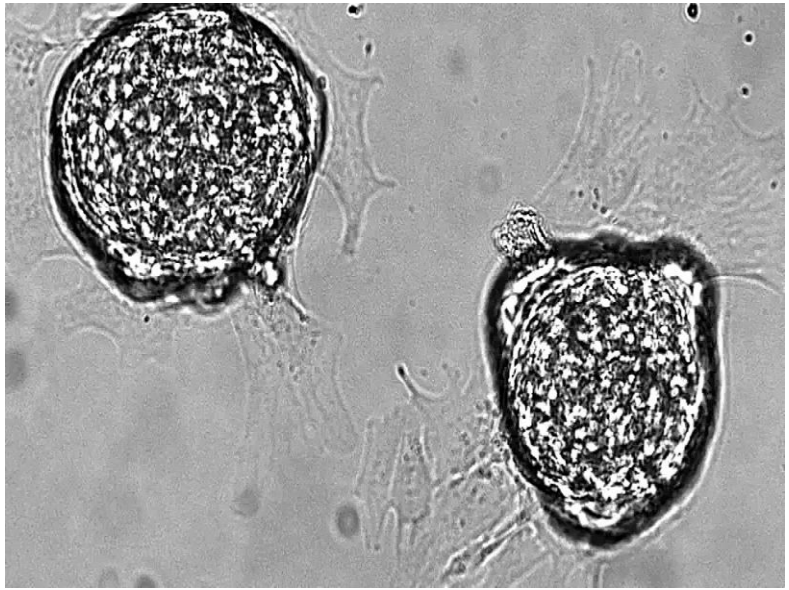
Synchronisation

Can we do it?

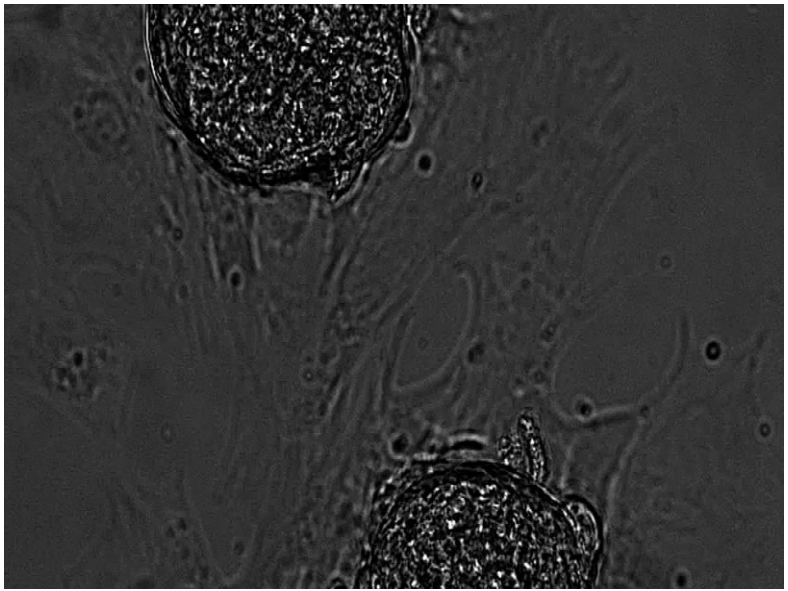
Real time drug arrhytmogenicity monitoring – conductive defects

2 beating cardiac organoids (200um apart) – fully synchronized beating

asynchronous



synchronized twins

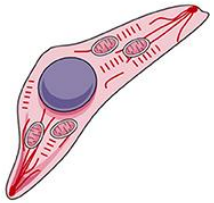


Pivato et al. ACB 2022
AFM Biomed Naogya-Okazaki 2022

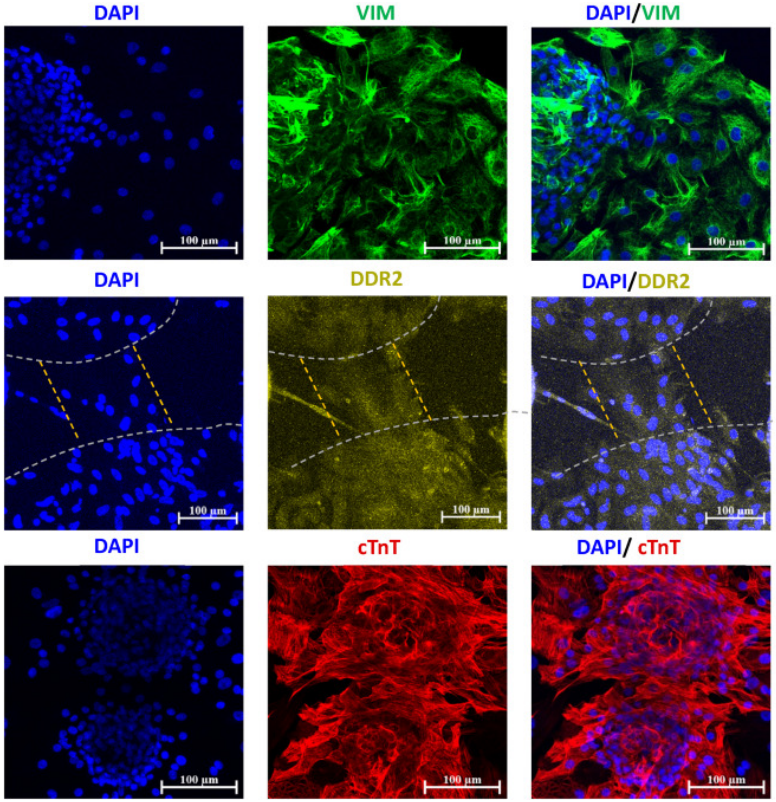
Real time drug arrhytmogenicity monitoring – conductive defects

2 beating cardiac organoids (200um apart) – fully synchronized beating

myofibroblasts



cardiomyocytes



DDR2 Discoidin Domain Receptor

VIM Vimentin

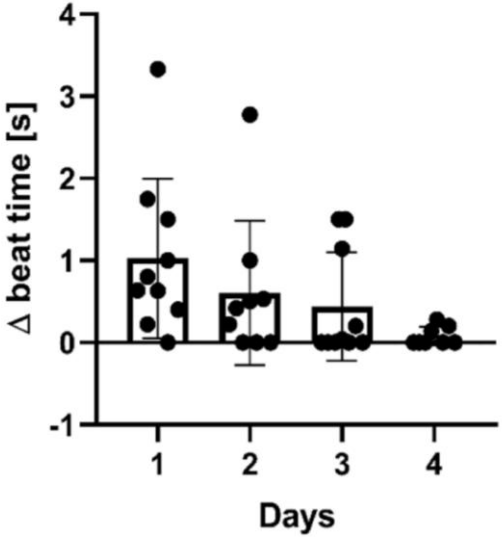
cTnT Cardiac Troponin T

Pivato et al. ACB 2022
AFM Biomed Naogya-Okazaki 2022

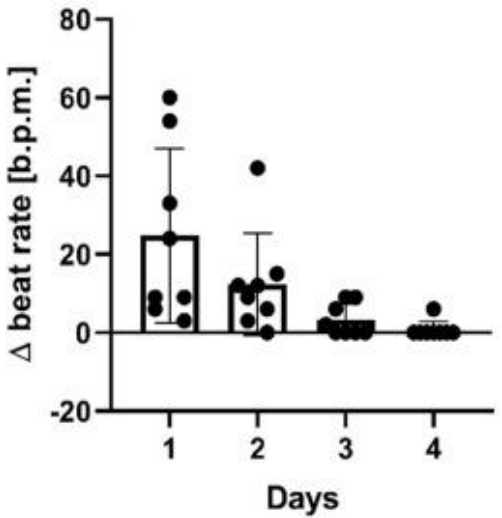
Real time drug arrhytmogenicity monitoring – conductive defects

Full synchronization by myofibroblast overgrowt in 4 days

Difference in beat frequency in time



Difference in beat rate in time

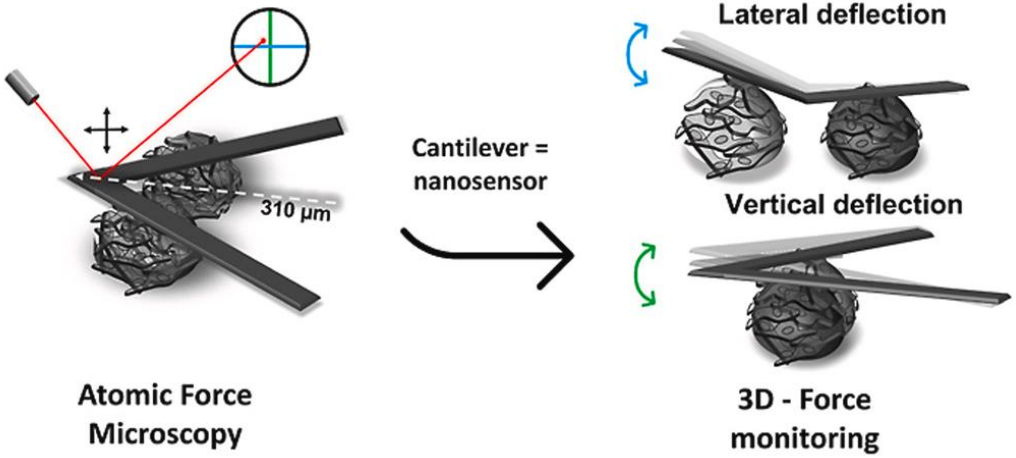


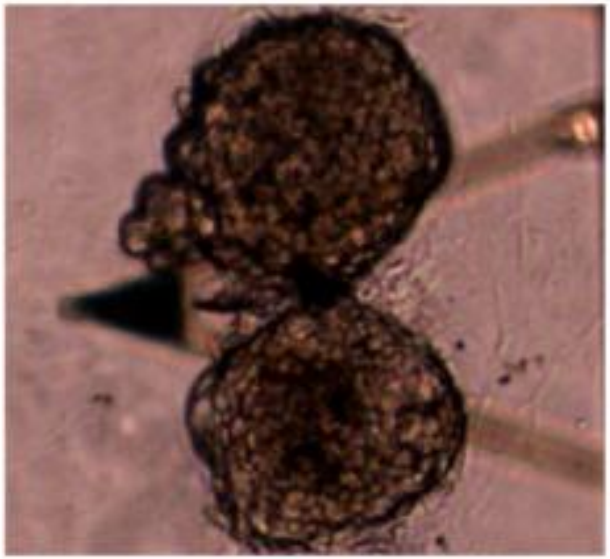
Evaluated by ImageJ/Fiji macro Musclemotion

Pivato et al. ACB 2022
AFM Biomed Naogya-Okazaki 2022

Real time drug arrhytmogenicity monitoring – conductive defects

Atomic Force Microscope biosensor using couples cardiac organoid twin structure



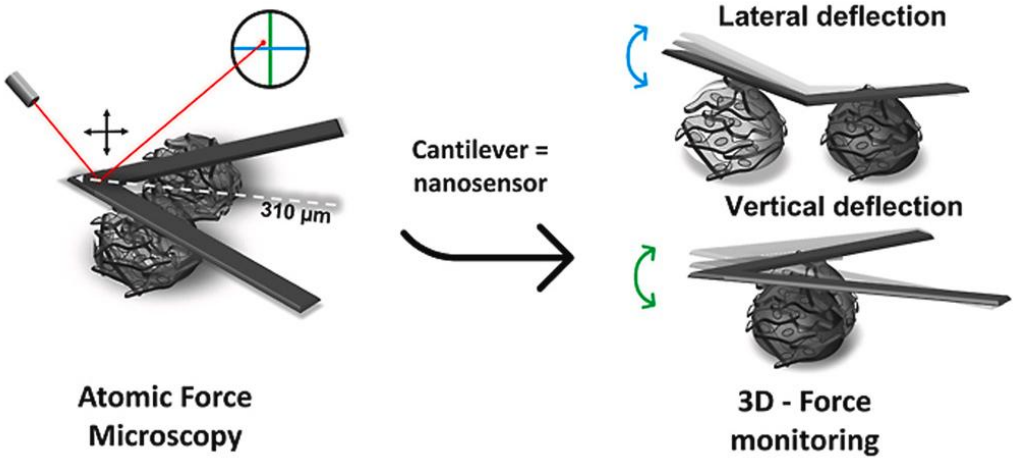


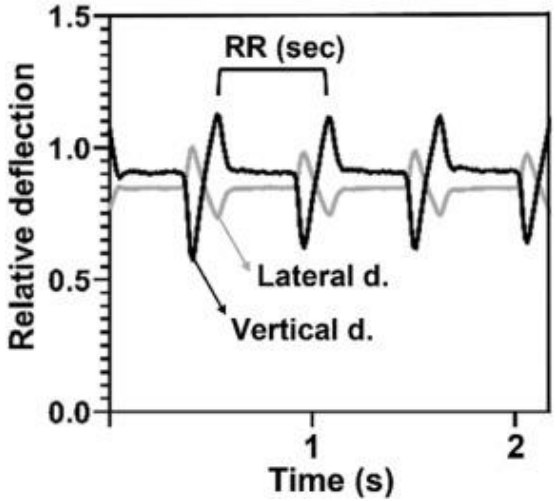
Pivato et al. ACB 2022
AFM Biomed Naogya-Okazaki 2022

Real time drug arrhytmogenicity monitoring – conductive defects

Atomic Force Microscope biosensor using couples cardiac organoid twin structure

Vertical and lateral deflection „synchronized“

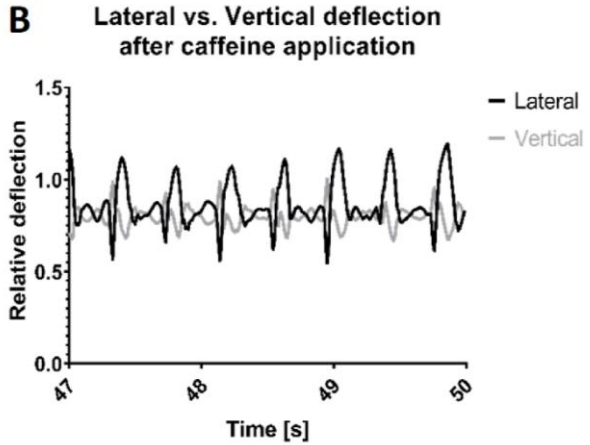


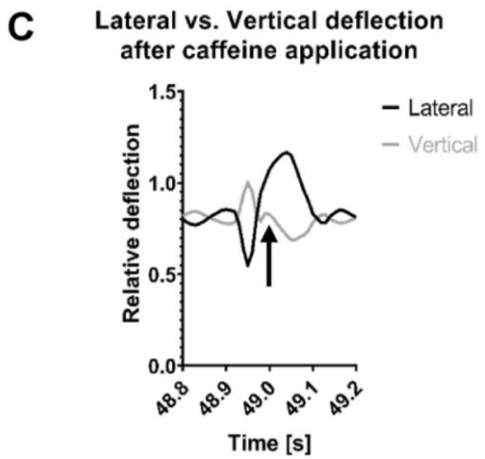


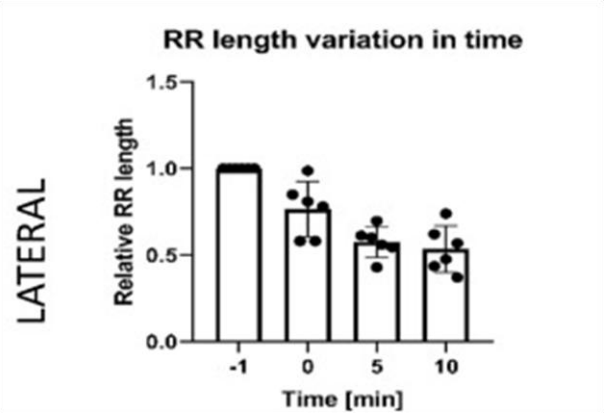
Pivato et al. ACB 2022
AFM Biomed Naogya-Okazaki 2022

Real time drug arrhytmogenicity monitoring – conductive defects

Caffeine conductive arrhytmogenicity







Vertical and lateral deflection „de-synchronized“

Pivato et al. ACB 2022
AFM Biomed Naogya-Okazaki 2022

Real time drug arrhytmogenicity monitoring – conductive defects

CONCLUSIONS

The lateral and vertical deflection peaks were synchronized in dual CMs cluster model.

Caffeine affected the synchronization of vertical and lateral displacement and produced independent lateral and vertical deflections.

Defects in signal spreading through the bridge resulting in the irregular beat of the two clusters

Serves as a novel model for advanced drug screening and disease-drug interaction.

Thank you for your attention..

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