

COMMENTARY TO HABILITATION THESIS¹

Cardiomyocyte (CM) contraction and relaxation are functional parameters that are fundamentally altered in most cardiac pathologies. The phenotypic characteristics of these myocardial diseases range from entirely asymptomatic forms to symptomatic heart failure and death resulting from malignant tachycardias (Martinez et al. 2021), with the underlying cause often residing at the cellular and genetic levels (Aistrup et al. 2009; Stienen 2015). Understanding the genetic-phenotypic associations of hereditary cardiac diseases enables more effective risk stratification (Stroeks et al. 2025), which improves clinical surveillance and risk management for both the patient and family members at risk of preventable cardiovascular morbidity and mortality (Carrick et al. 2024). Particularly in the era of precision medicine, this understanding provides the first opportunities for targeted therapy—for example, regarding a patient's ability to metabolize propafenone (Smíšek et al. 2025) or using Recursive Piecewise Data Assimilation (RPDA) to quantify alterations in ion channels, demonstrating the potential of this method as a drug toxicity counter-screen (Wells et al. 2025).

Since 1998, human cellular models became widespread, initially derived from human embryonic stem cells (hESC) (Thomson et al. 1998) and in the next decade in patient-specific induced pluripotent stem cells (hiPSC) (Takahashi and Yamanaka 2006), collectively called human pluripotent stem cells (hPSC). The cardiomyocytes differentiation methods quickly developed in co-culture, monolayers or clusters (three dimensional embryoid bodies, EBs) (reviewed in (Acimovic et al. 2014)). Resulting progenitor and maturing cells are important in drug screening and disease modeling (reviewed in (Svobodova et al. 2021)). The analysis of cardiomyocytes contractility phenotype represents an initial phase for further mechanical studies relevant for drug development and disease modeling (Martin Pesl et al. 2023; Caluori et al. 2019). Beating force and contraction frequency of the CMs contractions can be recorded by micromechanical transducers, a platform for cell-based biosensors (CBB). Strategies involving CBBs represent a powerful tool to understand and specifically treat human heart diseases.

We have constructed a sensitive setup that involves human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs), combined with an atomic force microscopy (AFM) force sensing platform, to measure biomechanical properties (absolute values of contractile forces together with the beat rate) in complex conditions and cellular models (Pesl et al. 2013) and continuously develop this setup for clinically relevant purposes.

The habilitation thesis presents analysis of cardiomyocytes by atomic force microscopy, the role of specific drugs, genetic and molecular factors. Alteration of electrical and mechanical function of cardiomyocytes are described in cardiac organoid models, which closely mimic the complexity of human heart development and give exceptional insights into sarcomere specification, gene expression regulation, and cardiac morphogenesis.

¹ The commentary must correspond to standard expectations in the field and must include a brief characteristic of the investigated matter, objectives of the work, employed methodologies, obtained results and, in case of co-authored works, a passage characterising the applicant's contribution in terms of both quality and content.

Aims of habilitaion thesis are to:

- 1 Describe the force and frequency of contractions of a cluster of cardiomyocytes differentiated from stem cells.
- 2 To measure absolute contractile force values along with contraction frequency in cellular models.
- 3 To extend analysis beyond mechanics and include other related modalities
- 4 To apply technology in genetic models
- 5 To apply technology in pharmacological analysis
- 6 To apply technology in specific area of arrhythmology – conductive arrhythmias

Employed Methodologies

The three basic methods utilized across the research works are briefly summarized in Table 1. This methodological framework consists of Atomic Force Microscopy (AFM) for recording mechanocardiograms, Microelectrode Arrays (MEA) for electrophysiological recordings, and Calcium Imaging for the direct monitoring of intracellular calcium transients.

METHOD	WHAT IT MEASURES	KEY ADVANTAGES
AFM (MECHANOCARDIOGRAMS)	Contractile force, beat rate, relaxation time	High sensitivity (200 pN), real-time mechanical readout
MEA (ELECTROPHYSIOLOGY)	Field potentials, beat frequency, arrhythmia risk	Correlates electrical excitation with mechanical output
CALCIUM IMAGING	Intracellular Ca ²⁺ transients (timing & amplitude)	Direct insight into excitation–contraction coupling

Tab.: 1. Overview of utilized methods

Atomic force microscopy

AFM-based biosensor noninvasively characterizes the contractile mechanics of clusters of human pluripotent stem cell–derived cardiomyocytes. Uncoated silicon-nitride cantilevers were mounted in a fluidic chamber at physiological conditions (~37 °C, buffered culture medium). Setup record continuous real-time mechanical force-time curves—termed mechanocardiograms (MCGs). These capture beat-to-beat contraction force, beat rate, and relaxation dynamics. This setup has excellent sensitivity, with a detection limit down to ~200 pN and ~20 nm vertical displacement, providing robust, low-computational readouts. To optimize signal accuracy across the entire syncytium, cantilever parameters such as feedback gain and spring constant (optimized around 6 nN) were fine-tuned (Pribyl, Pesl et al. 2019), and it was demonstrated that uncoated (non-coated) cantilevers provide enhanced thermal stability for prolonged measurements lasting hours to days. This method also enables pharmacological testing—for instance, measuring inotropic responses to isoproterenol, aminophylline, salbutamol, or β -blockers—thereby translating mechanical changes into quantifiable indicators of drug effect (Kabanov et al. 2024, Klimovic et al. 2022).

Microelectrode array

To complement the mechanical insight, the AFM platform was integrated with microelectrode arrays (MEAs)—exactly aligning beating clusters of cardiomyocytes cultured atop MEA chips with simultaneous cantilever probing. In this dual-modal electromechanical system,

extracellular field potentials (FPs)—including beat frequency, waveform morphology, and arrhythmic events—were captured via MEA electrodes, while the AFM cantilever recorded mechanical outputs from the same biological structure.

This configuration enabled precise correlation between electrical excitation (FP signals) and mechanical contraction, offering a richer, multidimensional phenotype readout. Optical–mechanical synchronization involved inverted optical microscopy enabling simultaneous visualization and data alignment for the two modalities.

Calcium Imaging

The third methodological pillar is the combination of AFM with optical calcium imaging, enabling direct monitoring of intracellular calcium dynamics alongside mechanical contraction. In their conference abstract, Pešl et al. reported the first simultaneous recordings of mechanocardiograms (via AFM) and local calcium wave dynamics (likely using fluorescent Ca^{2+} -sensitive dyes) in cardiomyocyte clusters—delivering rich data on excitation-contraction coupling within embryoid bodies (EBs) (Odstrcilik et al. 2015)

Signal processing employed ensemble empirical mode decomposition (EEMD), which they found optimally balanced signal-to-noise ratio and minimized distortion. They validated the system by applying caffeine—a known modulator of calcium handling—and observed the expected physiological effects: ~5.5 % increase in calcium transient duration and ~14.5 % increase in contraction amplitude. This AFM- Ca^{2+} integration thus empowers real-time correlation between Ca^{2+} spike timing, amplitude, and mechanical force generation. (Cmiel 2017).

Obtained Results

The significant findings of the thesis include:

- Atomic Force Microscopy (AFM) is novel high-fidelity nanomechanical transducer with a sensitivity down to ~200 pN, using non-coated silicon nitride cantilevers with a 5–6 nN setpoint provides the thermal stability necessary for continuous, non-invasive recordings of cardiac activity lasting up to 8 hours
- The platform achieved the first-ever synchronous recording of extracellular field potentials (via MEA) and mechanical force (via AFM) in 3D cardiac models. This integration allowed for the quantification of the electro-mechanical delay (EMD), providing a novel and critical parameter for assessing excitation-contraction coupling in vitro.
- By utilizing patient-specific hiPSCs and CRISPR/Cas9-edited lines, the human Duchenne Muscular Dystrophy (DMD) phenotype was described. Key findings included a distinctive signature of hypocontractility, bradycardia, increased heart rate variability, and impaired calcium handling marked by diastolic calcium leaks
- Research on aminophylline identified two parallel arrhythmogenic actions: a "deterministic" concentration-dependent chronotropic effect (sinus tachycardia) and a "stochastic" concentration-independent occurrence of rhythm irregularities (resembling atrial fibrillation). The study of the salbutamol-aminophylline combination revealed that salbutamol acts as an anti-arrhythmic agent by mitigating aminophylline-induced irregularities. This effect was mechanistically linked to the activation of nitric oxide synthase (NOS) via β 2-adrenergic receptors.
- The development of a dual 3D cardiac syncytium connected by a functional cellular bridge allowed for the detailed study of conduction disturbances. By utilizing lateral AFM deflection measurements, the biosensor detected the loss of synchrony and signal spreading defects characteristic of fibrillation-like behaviour.

Applicant's Contribution

Applicant initially performed cardiac organoid cultivation, gene editing and iPS line derivation and performed analytical methods, mainly Atomic force microscopy. Throughout all the works applicant analyzed the experimental data, including AFM data and imaging results. Since 2016 was applicant core part of conceptualization and Design and Developed the research hypothesis and designed the experimental approaches.

In later stages were supervised Ph. D. students along with Mgr. Jan Příbyl Ph. D. as head of AFM facility at CEITEC and Mgr. Vladimír Rotrekl Ph. D. as head of research group, thus both supervision and research direction is often shared as 50% or 30%. In two of presented papers is applicant first author, in one corresponding author. In this paper applicant supervised a team of researchers and coordinated collaborative efforts to ensure the successful completion of the project (NU20-06-00156 The effect of bronchodilator drugs on human cardiomyocytes).

Each publication is related to afore mentioned aim, number 5 and six are both related to Aim 5 "To apply technology in pharmacological analysis".

- 1) Describe the force and frequency of contractions of a cluster of cardiomyocytes differentiated from stem cells.

[1] **PESL, Martin**, Jan PRIBYL, Ivana ACIMOVIC, Alexandra VILOTIC, Sarka JELINKOVA, Anton SALYKIN, Alain LACAMPAGNE, Petr DVORAK, Albano C. MELI, Petr SKLADAL and Vladimír ROTREKL. Atomic force microscopy combined with human pluripotent stem cell derived cardiomyocytes for biomechanical sensing. *Biosensors and Bioelectronics* [online]. 2016, **85**, 751–757. ISSN 0956-5663. Available at: doi:10.1016/j.bios.2016.05.073

First author

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
30%	20%	50%	30%

- 2) To measure absolute contractile force values along with contraction frequency in cellular models.

[2] CALUORI, Guido, Jan PRIBYL, **Martin PESL**, Sarka JELINKOVA, Vladimír ROTREKL, Petr SKLADAL and Roberto RAITERI. Non-invasive electromechanical cell-based biosensors for improved investigation of 3D cardiac models. *Biosensors and Bioelectronics* [online]. 2019, **124–125**, 129–135. ISSN 0956-5663. Available at: doi:10.1016/j.bios.2018.10.021

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20%	30%	30%	50%

- 3) To extend analysis beyond mechanics and include other related modalities

[3] CALUORI, Guido, Jan PRIBYL, Vratislav CMIEL, **Martin PESL**, Tomas POTOČNAK, Ivo PROVAZNIK, Petr SKLADAL and Vladimír ROTREKL. Simultaneous study of mechanobiology and calcium dynamics on hESC-derived cardiomyocytes clusters. *Journal of Molecular Recognition* [online]. 2019, **32**(2, Article e2760). ISSN 0952-3499. Available at: doi:10.1002/jmr.2760

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
25%	20%	33%	50%

4) To apply technology in genetic models

[4] JELINKOVA, Sarka, Aleksandra VILOTIC, Jan PRIBYL, Franck AIMOND, Anton SALYKIN, Ivana ACIMOVIC, **Martin PESL**, Guido CALUORI, Simon KLIMOVIC, Tomas URBAN, Hana DOBROVOLNA, Vladimir SOSKA, Petr SKLADAL, Alain LACAMPAGNE, Petr DVORAK, Albano C. MELI and Vladimir ROTREKL. DMD Pluripotent Stem Cell Derived Cardiac Cells Recapitulate in vitro Human Cardiac Pathophysiology. *Frontiers in Bioengineering and Biotechnology* [online]. 2020, **8**(Article 535). ISSN 2296-4185. Available at: doi:10.3389/fbioe.2020.00535

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10%	20%	15%	30%

5a) To apply technology in pharmacological analysis

[5] KLIMOVIC, Simon, Martin SCUREK, **Martin PESL**, Deborah BECKEROVA, Sarka JELINKOVA, Tomas URBAN, Daniil KABANOV, Zdenek STAREK, Marketa BEBAROVA, Jan PRIBYL, Vladimir ROTREKL and Kristian BRAT. Aminophylline Induces Two Types of Arrhythmic Events in Human Pluripotent Stem Cell-Derived Cardiomyocytes. *Frontiers in Pharmacology* [online]. 2022, **12**(Article 789730). ISSN 1663-9812. Available at: doi:10.3389/fphar.2021.789730

Shared First Author

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10%	20%	30%	50%

5b) To apply technology in pharmacological analysis

[6] KABANOV, Daniil, Simon VRANA KLIMOVIC, Deborah BECKEROVÁ, Martin MOLCAN, Martin SCUREK, Kristian BRAT, Marketa BEBAROVA, Vladimir ROTREKL, Jan PRIBYL and **Martin PESL**. Salbutamol attenuates arrhythmogenic effect of aminophylline in a hPSC-derived cardiac model. *Scientific Reports* [online]. 2024, **14**(1, Article 27399). ISSN 2045-2322. Available at: doi:10.1038/s41598-024-76846-4

Corresponding Author

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10%	30%	30%	30%

6) To apply technology in specific area of arrhythmology – conductive arrhythmias

[7] PIVATO, Roberto, Simon KLIMOVIC, Daniil KABANOV, Filip SVERÁK, **Martin PESL**, Jan PRIBYL and Vladimir ROTREKL. hESC derived cardiomyocyte biosensor to detect the

different types of arrhythmogenic properties of drugs. *Analytica Chimica Acta* [online]. 2022, **1216**(Article 339959). ISSN 0003-2670. Available at: doi:10.1016/j.aca.2022.339959

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10%	25%	25%	30%