

Mechanobiology of *in vitro* assays: tackling prevailing challenges in pre- clinical drug development

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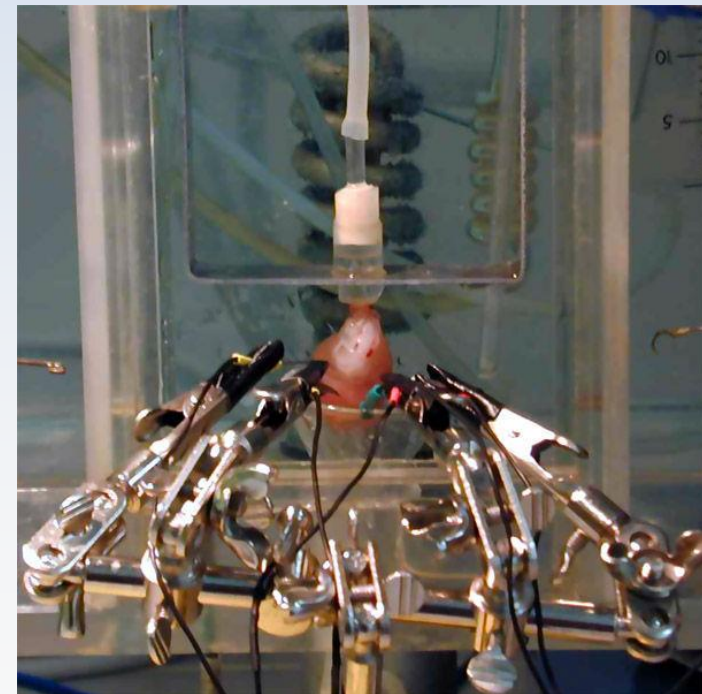
Content

- State-of-the-art in cardiac contractility measurement
- Mechanosensitivity of cardiomyocytes
 - Extracellular matrix
 - Active sensing
 - Gene expression
 - Ion channels
- The FLEXcyte 96 system
 - Introduction
 - Representative pharmacological data



State-of-the-Art

- The Langendorff Heart (1895) and related *ex vivo* techniques are still the „gold standard“ for contractility measurement
 - Con's:
 - Low predictivity
 - Low throughput
 - Labor-intensive
 - Pro's:
 - Physiologic responses
- Natural mechanical environment



Biomechanical View on Contractility

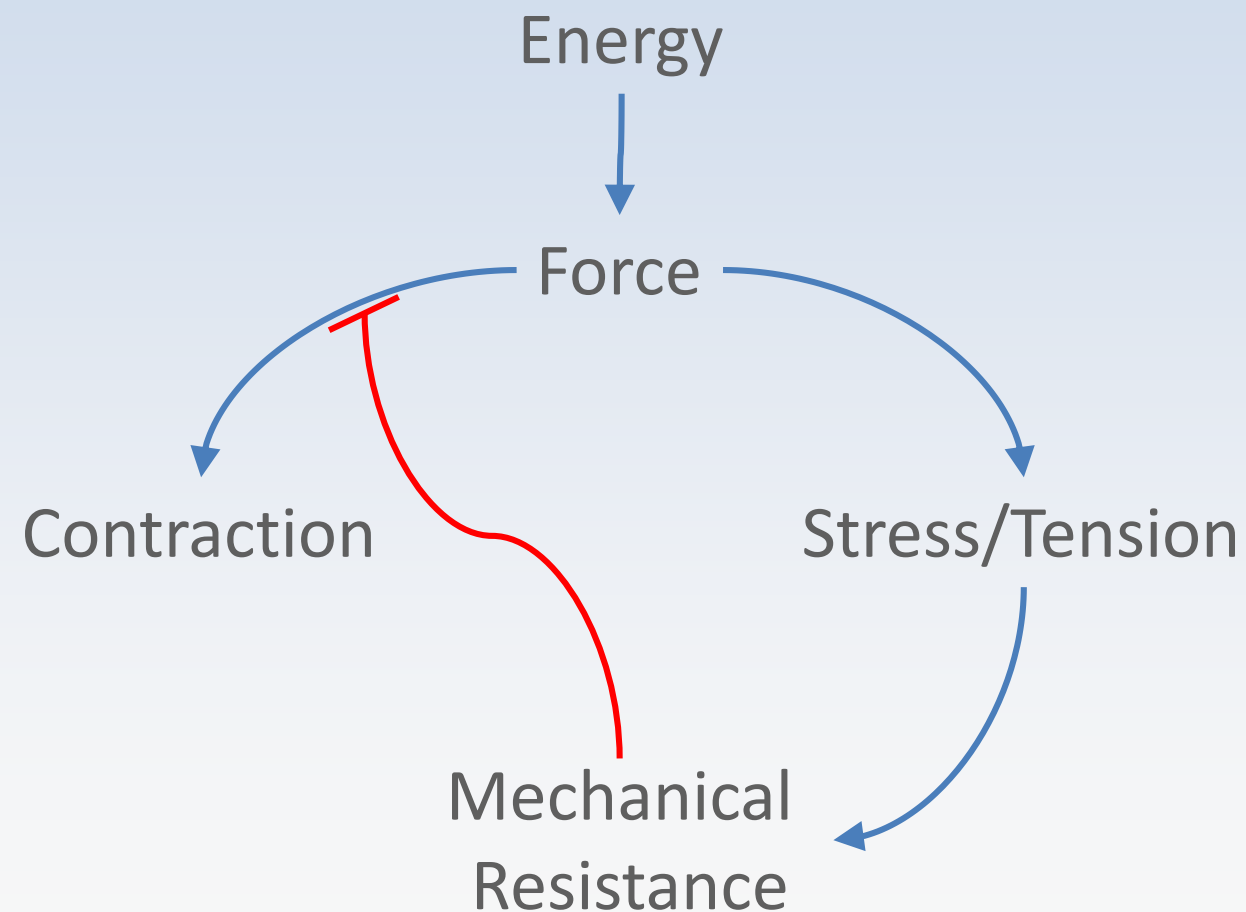
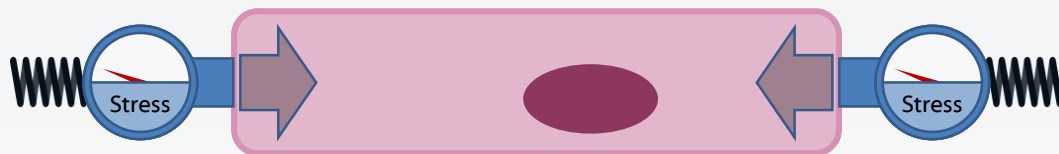
- Isotonic: Max Contraction



- Isometric: Max Stress

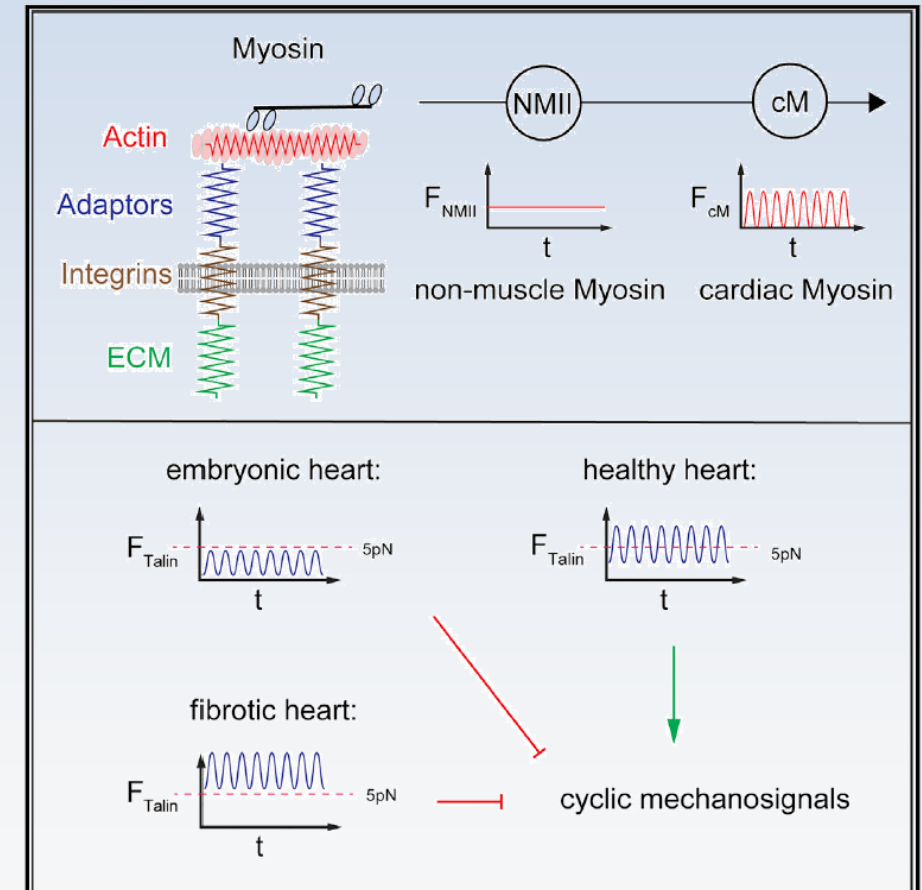


- Auxotonic: Physiological Relationship



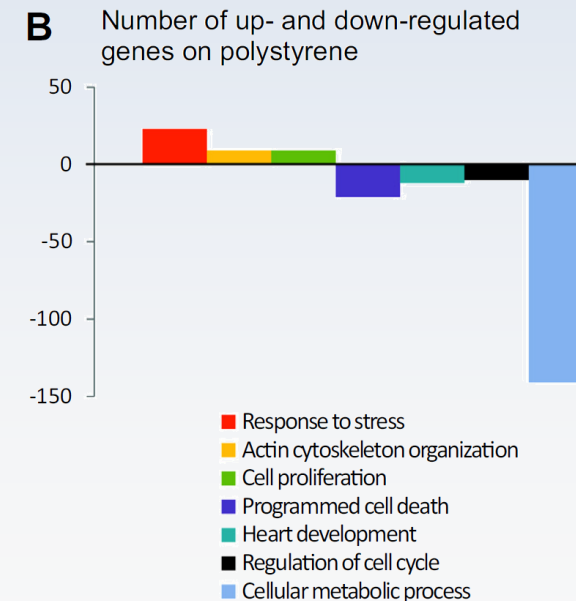
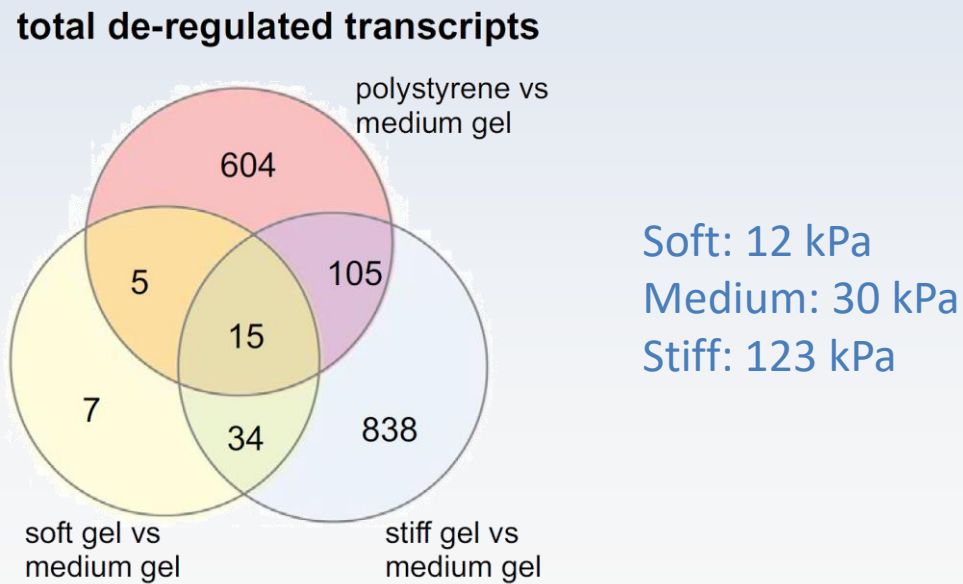
Mechanosensitivity: Active Sensing

- Cardiomyocytes use deformable adaptor proteins to sense:
 - Fast muscle myosin contraction
 - Slow non-muscle myosin contraction
- Deformation of different adaptor proteins regulate differential protein kinase pathways



Mechanosensitivity: Gene Expression

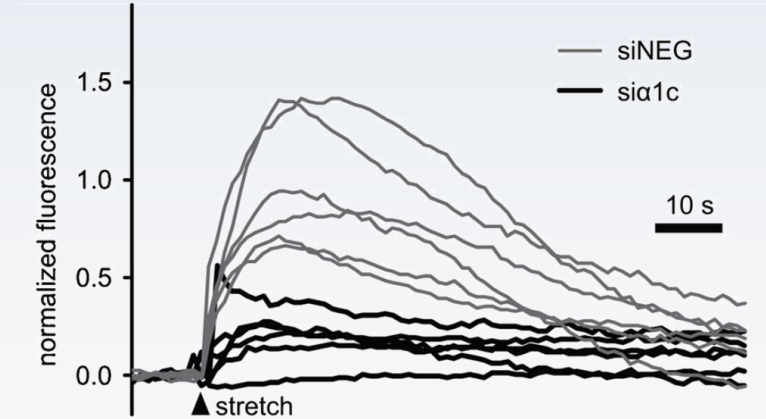
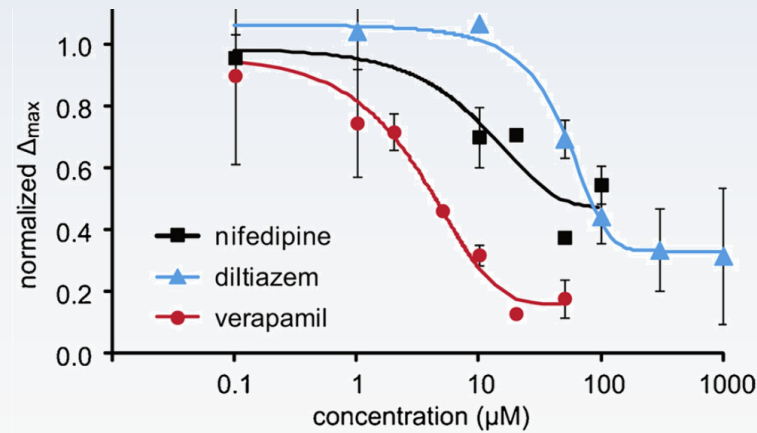
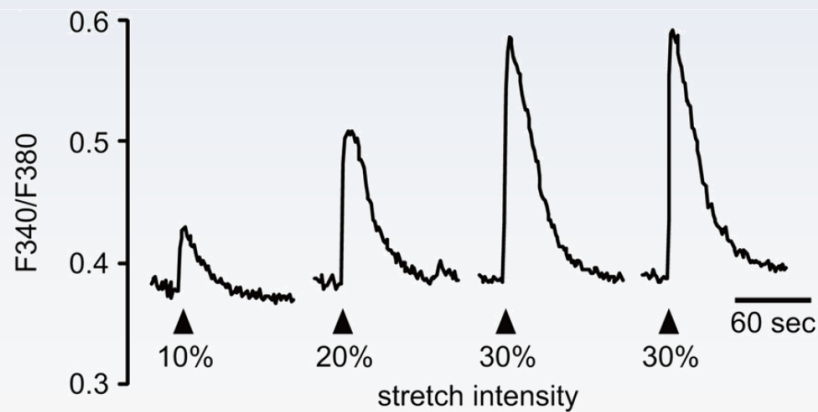
- Cardiomyocytes respond to unphysiologic matrix stiffness with drastic transcriptional de-regulation
- Metabolic processes are predominantly de-regulated on polystyrene



Heras-Bautista *et al.*: Cardiomyocytes facing fibrotic conditions re-express extracellular matrix transcripts.
 Acta Biomaterialia 89 (2019) 180-192

Mechanosensitivity: Ion Channels

- Increase of intracellular Ca^{2+} level is evoked by mechanical stimulation (uniaxial stretching)
- Magnitude of Ca^{2+} increase depends on stimulation intensity
- L-type calcium channels contribute to this effect



Takahashi et al.: L-type calcium channel modulates mechanosensitivity of the cardiomyocyte cell line H9c2.
Cell Calcium 79 (2019) 68-74

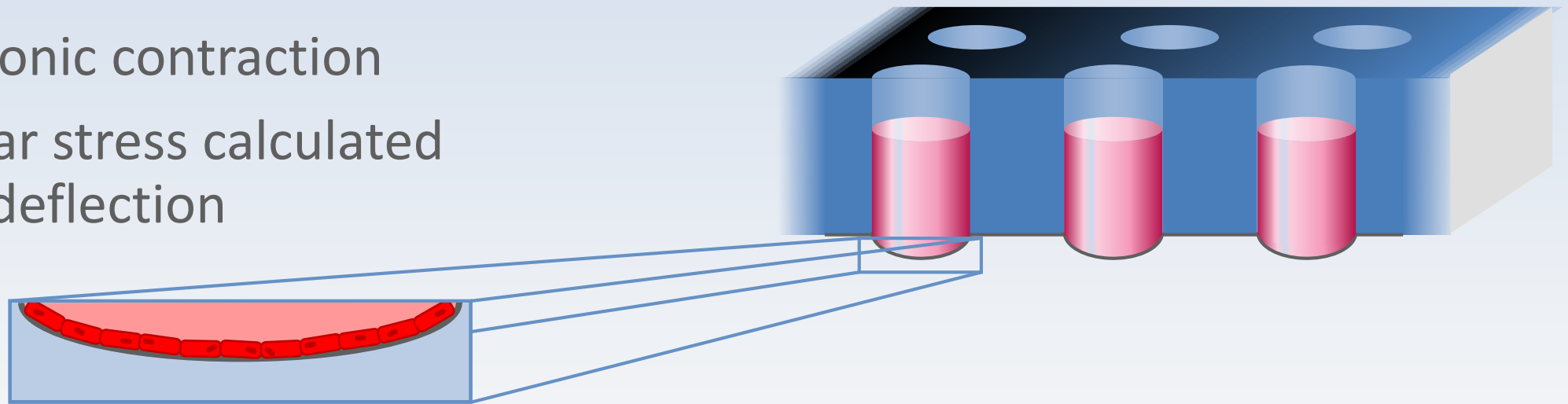
Challenges

To replace animal-based, *ex vivo* methods with human stem cell-derived *in vitro* models, the assay technology needs to combine:

- Predictivity / physiological relevance
- Throughput
- Cost-effectiveness

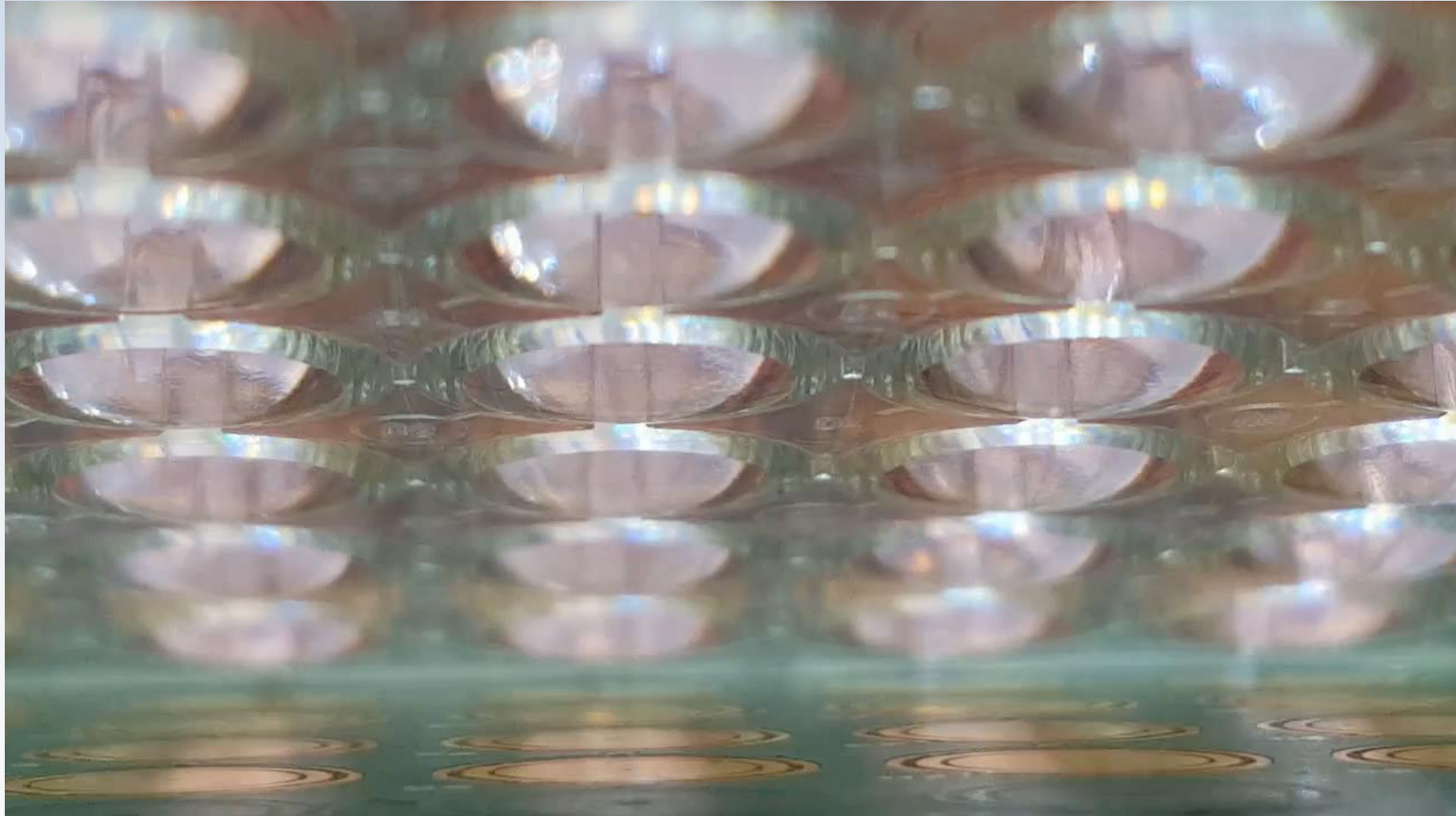
The FLEXcyte 96 System

- Ultra-thin silicone substrate
- Adjustable mechanical environment
- Auxotonic contraction
- Cellular stress calculated from deflection



$$\sigma = \frac{p}{h} \cdot \frac{r^2}{4s} \cdot \left(1 + \frac{h^2}{r^2} \right)$$

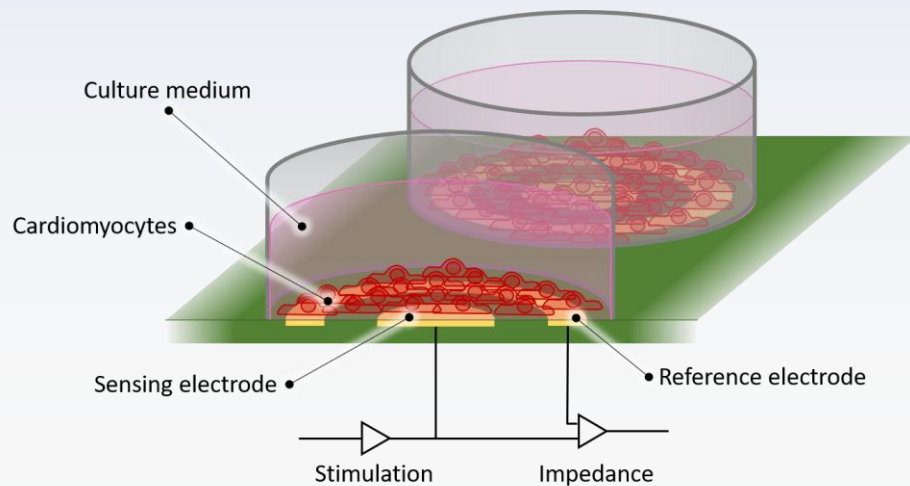
The FLEXcyte 96 System



The FLEXcyte 96 System

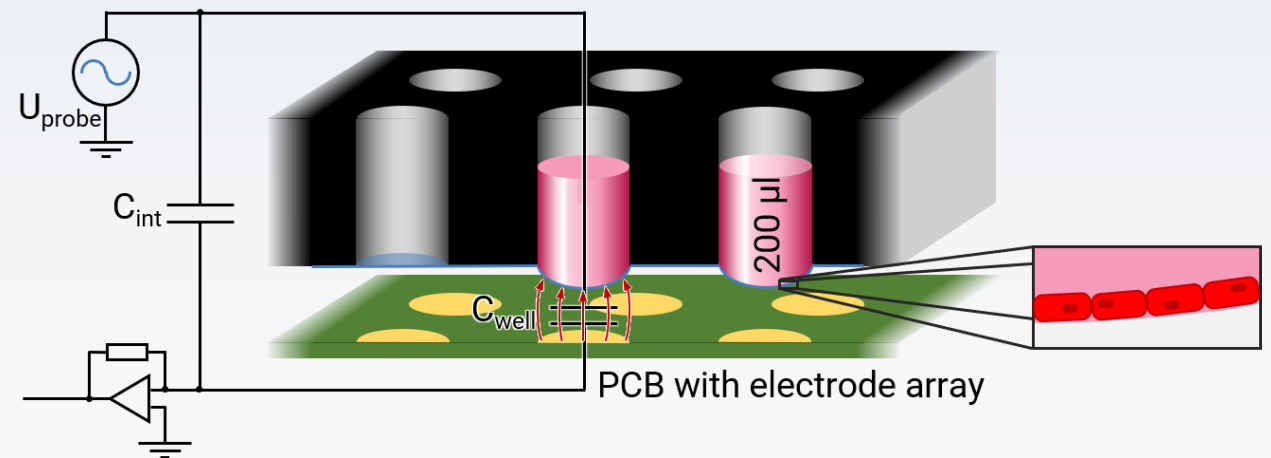
Impedance

- Infinitely stiff substrate
- Isometric contraction
- Parameter: Cellular shape



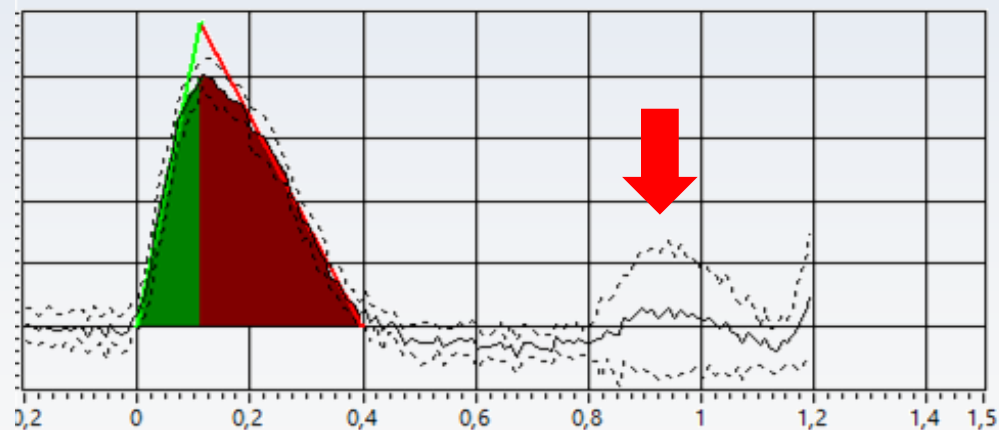
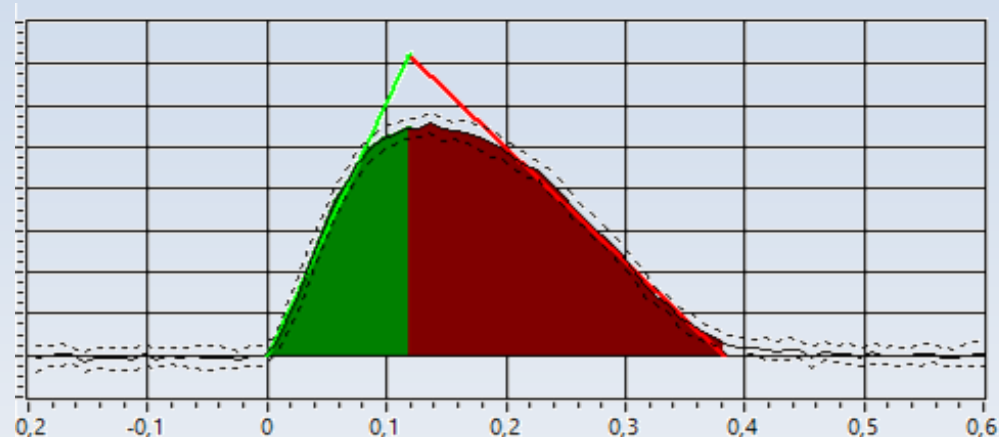
FLEXcyte 96

- Physiologic stiffness
- Auxotonic contraction
- Parameter: Contraction



The FLEXcyte 96 System

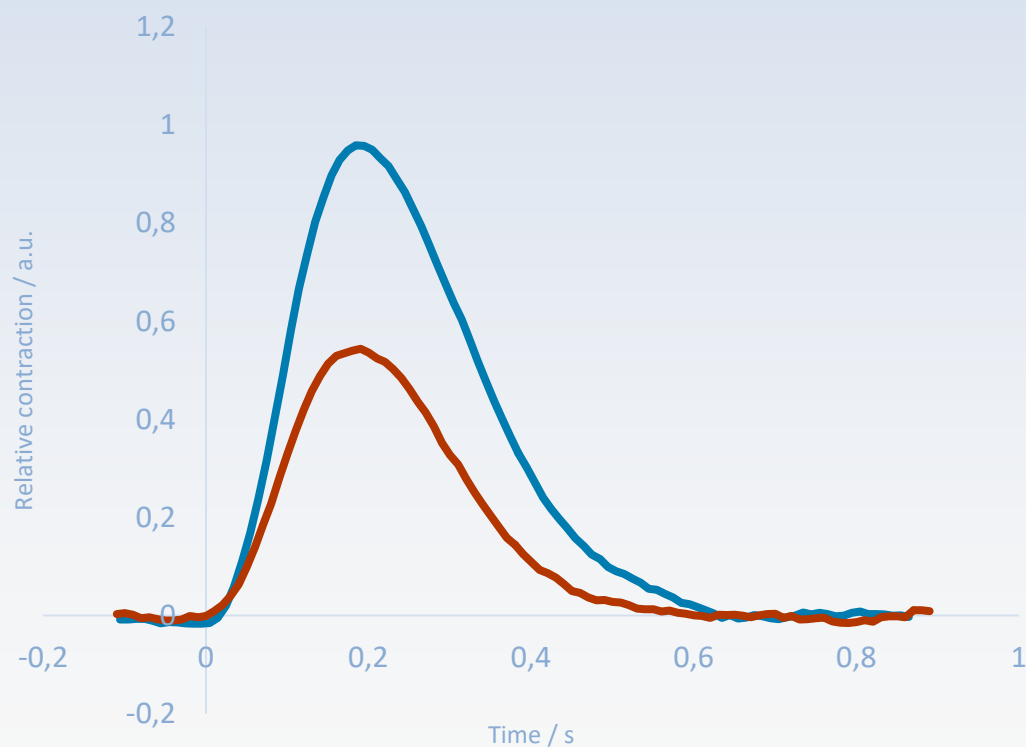
- Amplitude
- Durations
- Slopes
- Integrals
- Frequency
- Arrhythmic Events



Pharmacology: Negative Inotropes

Blebbistatin

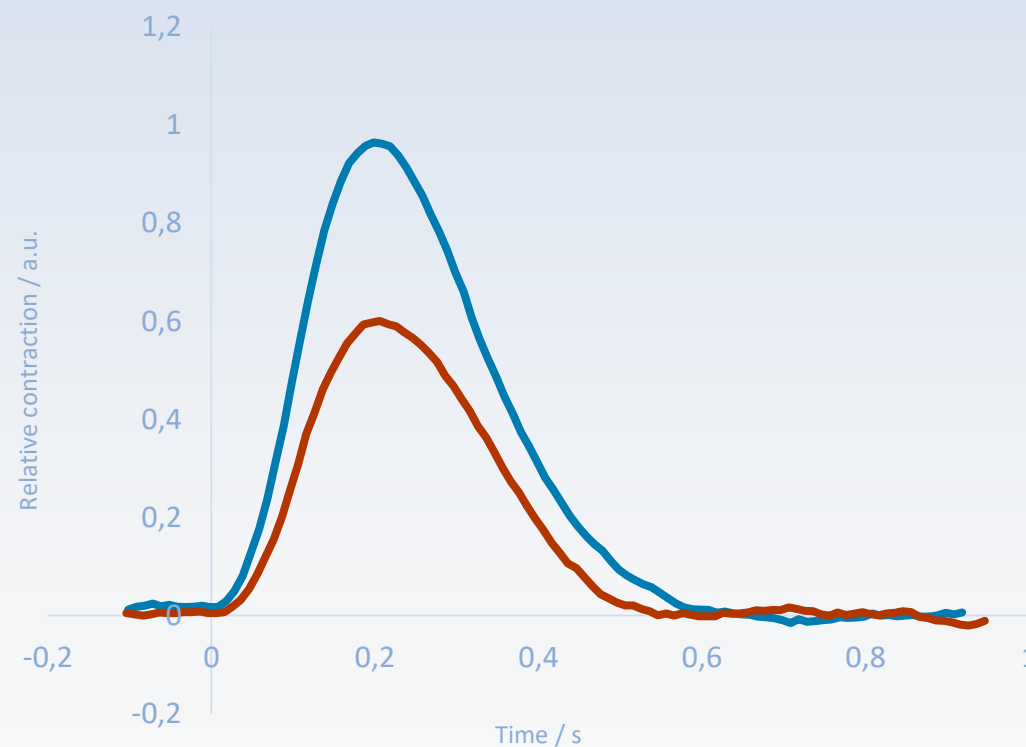
Control 100 nM



iCell Cardiomyocytes², FCDI

Nifedipine

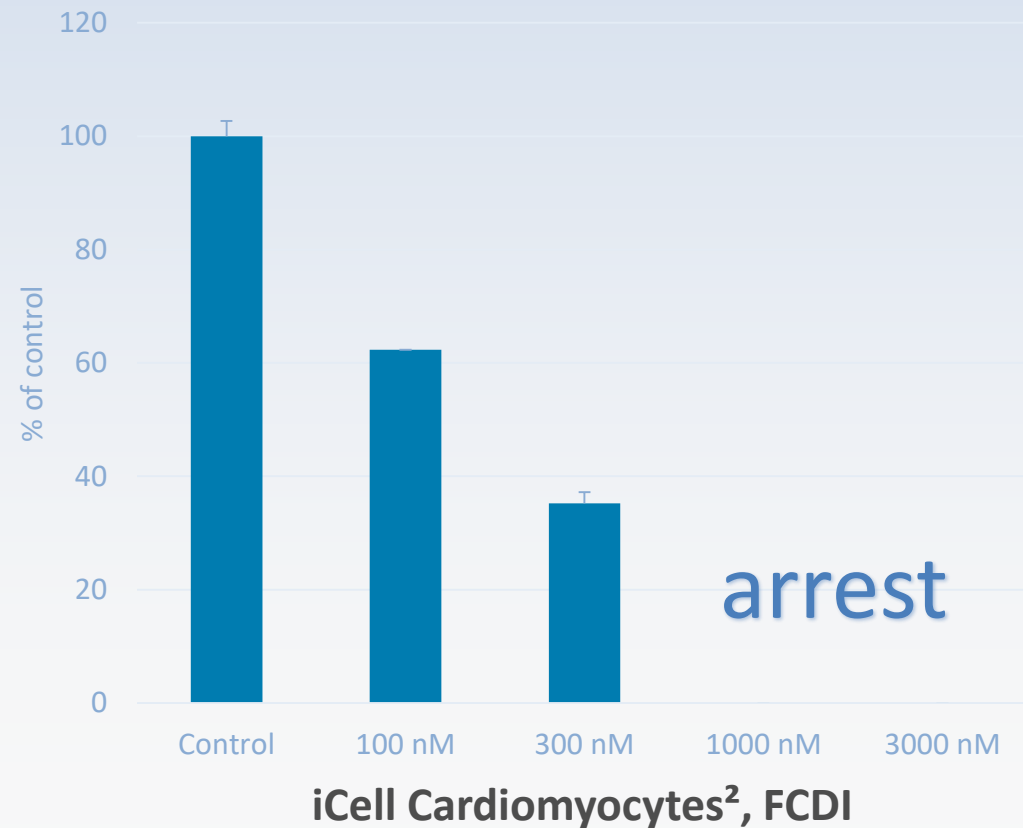
Control 30 nM



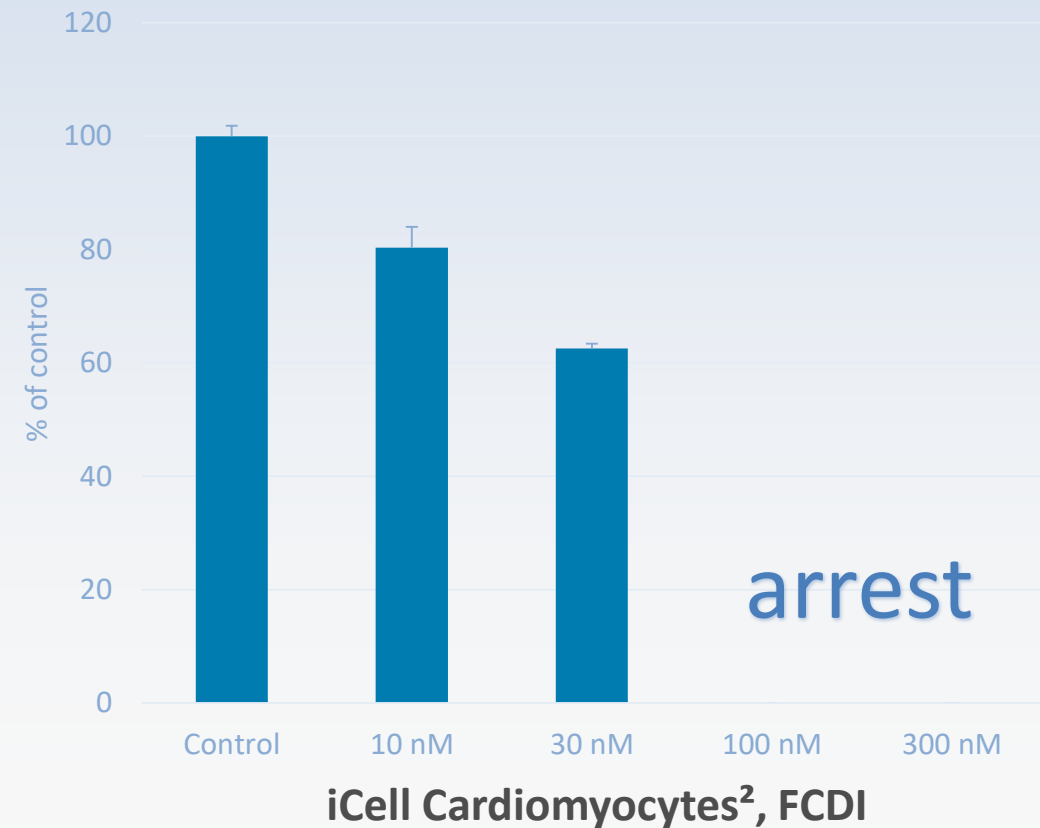
iCell Cardiomyocytes², FCDI

Pharmacology: Negative Inotropes

Blebbistatin



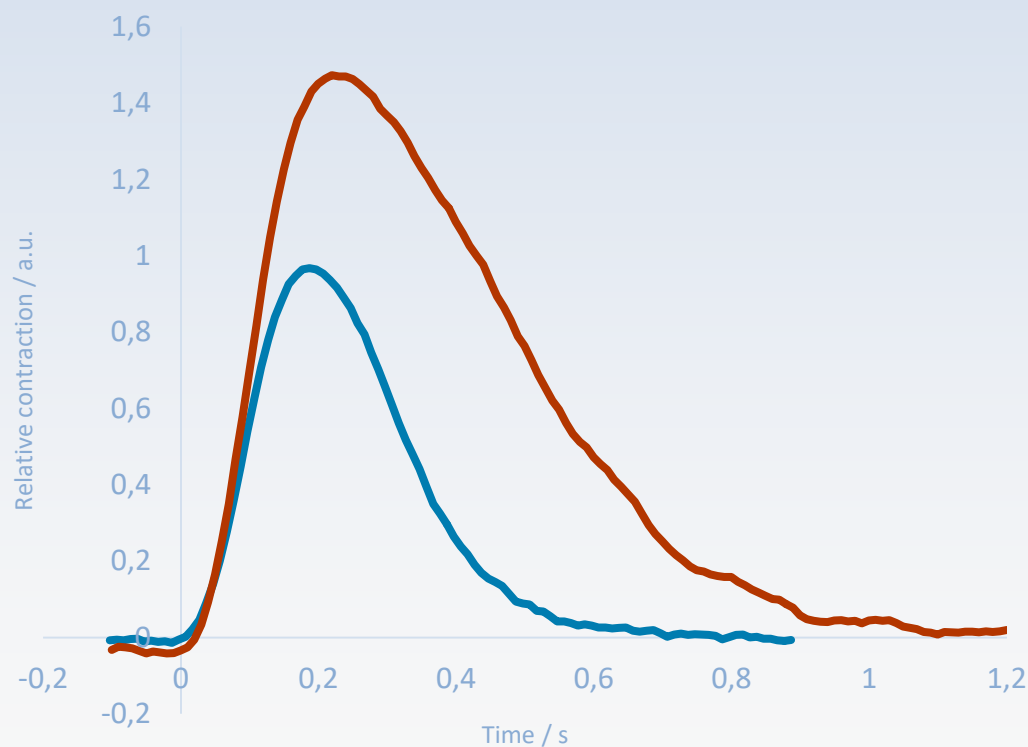
Nifedipine



Pharmacology: Positive Inotropes

S-Bay K8644

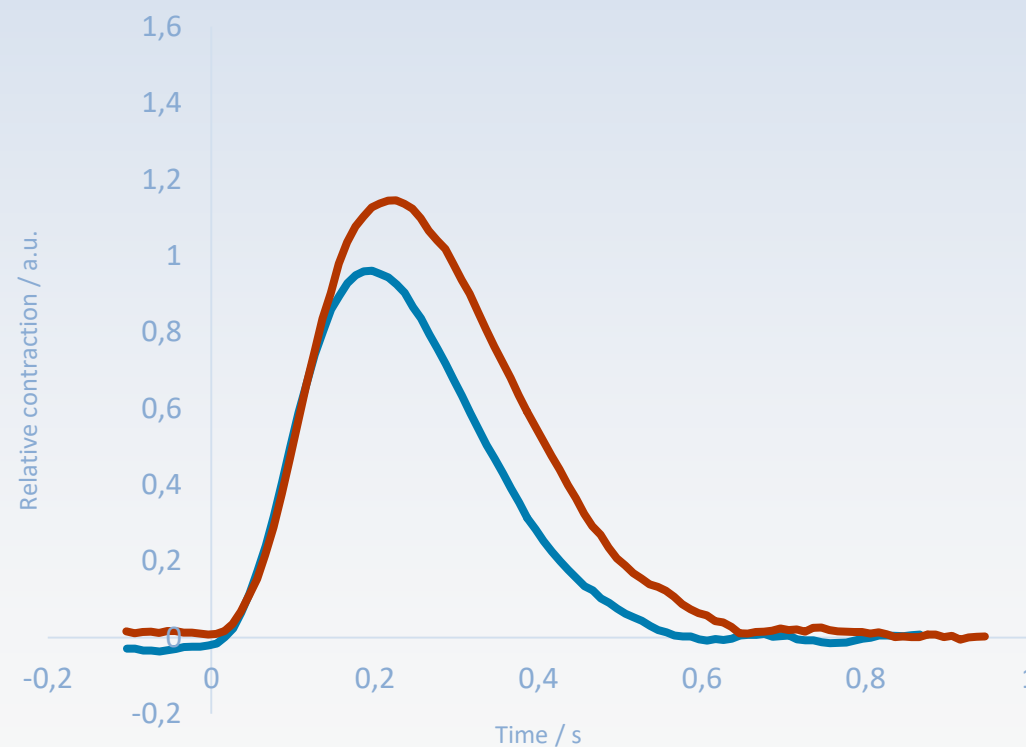
Control 10 nM



iCell Cardiomyocytes², FCDI

Isoproterenol

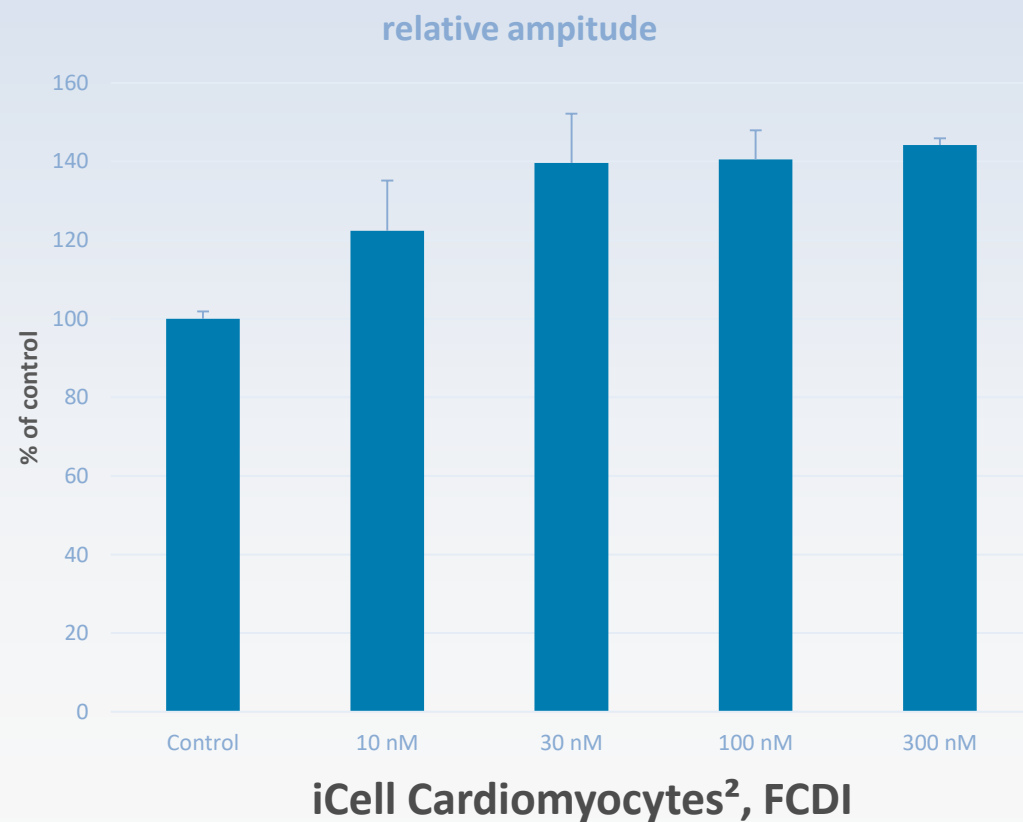
Control 10 nM



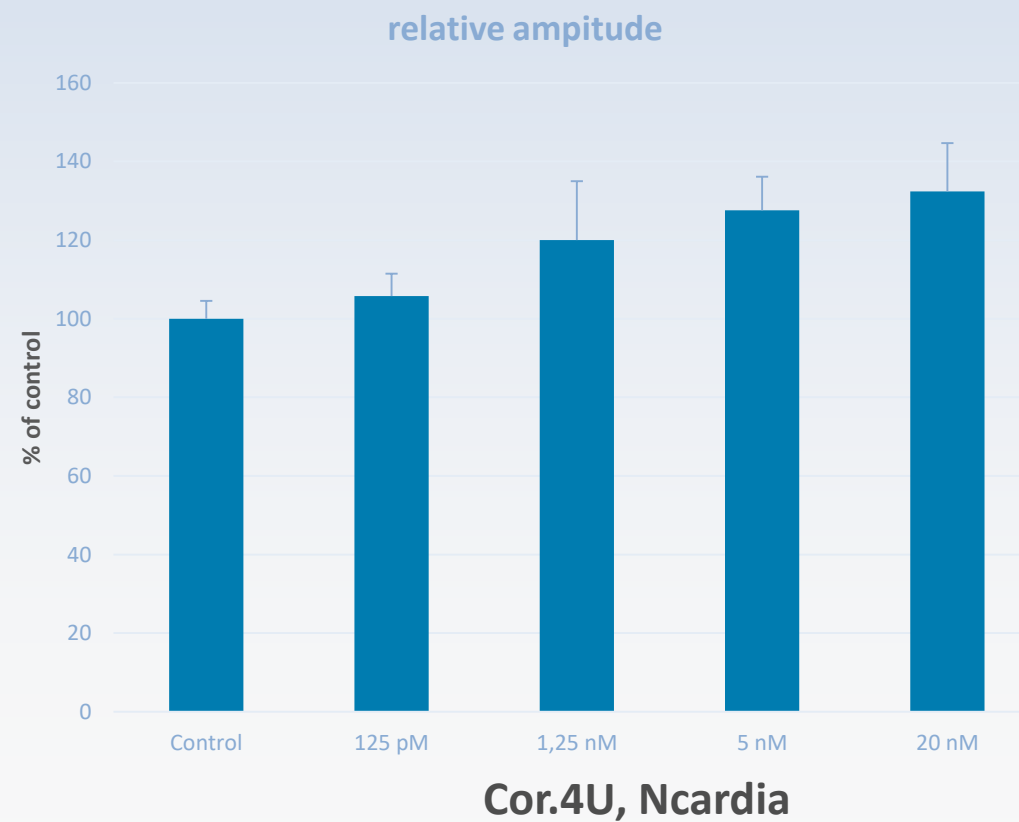
Cor.4U, Ncardia

Pharmacology: Positive Inotropes

S-Bay K8644

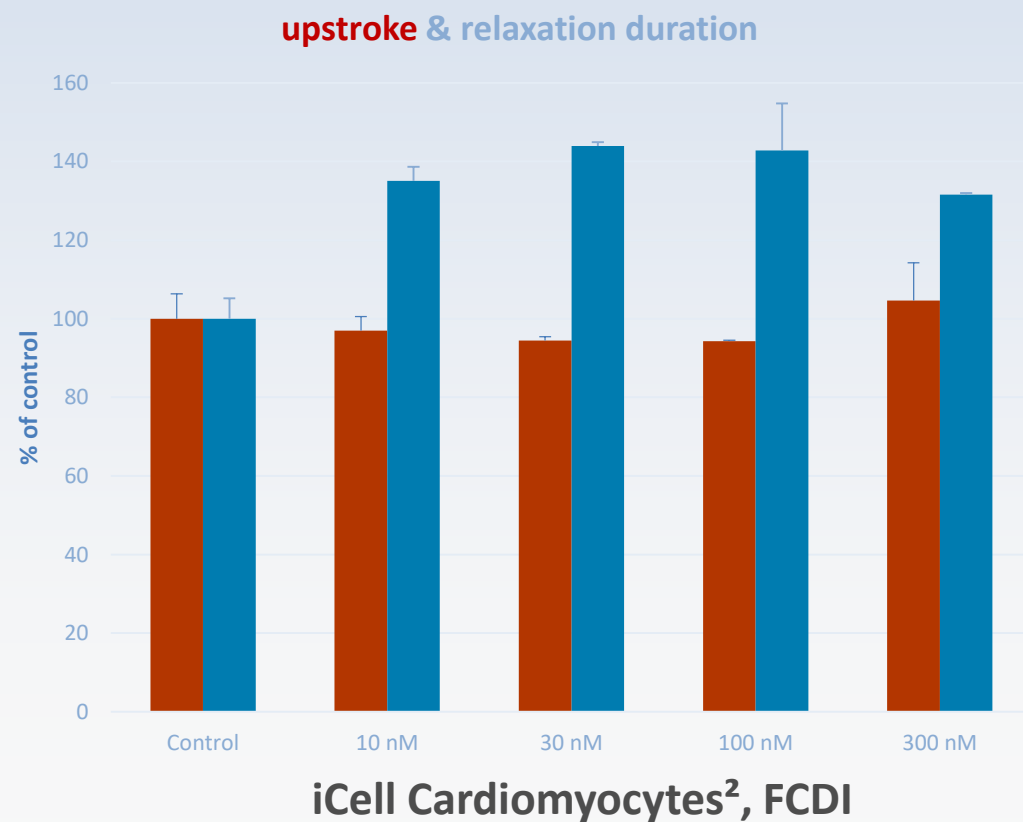


Isoproterenol

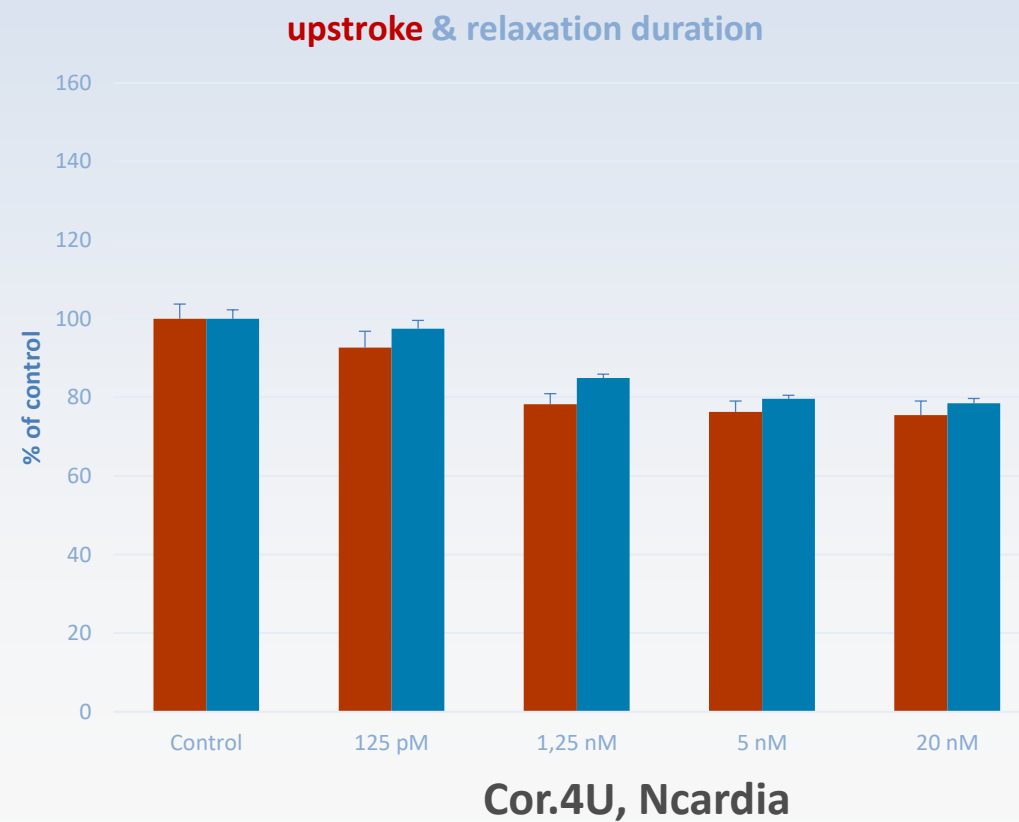


Pharmacology: Positive Inotropes

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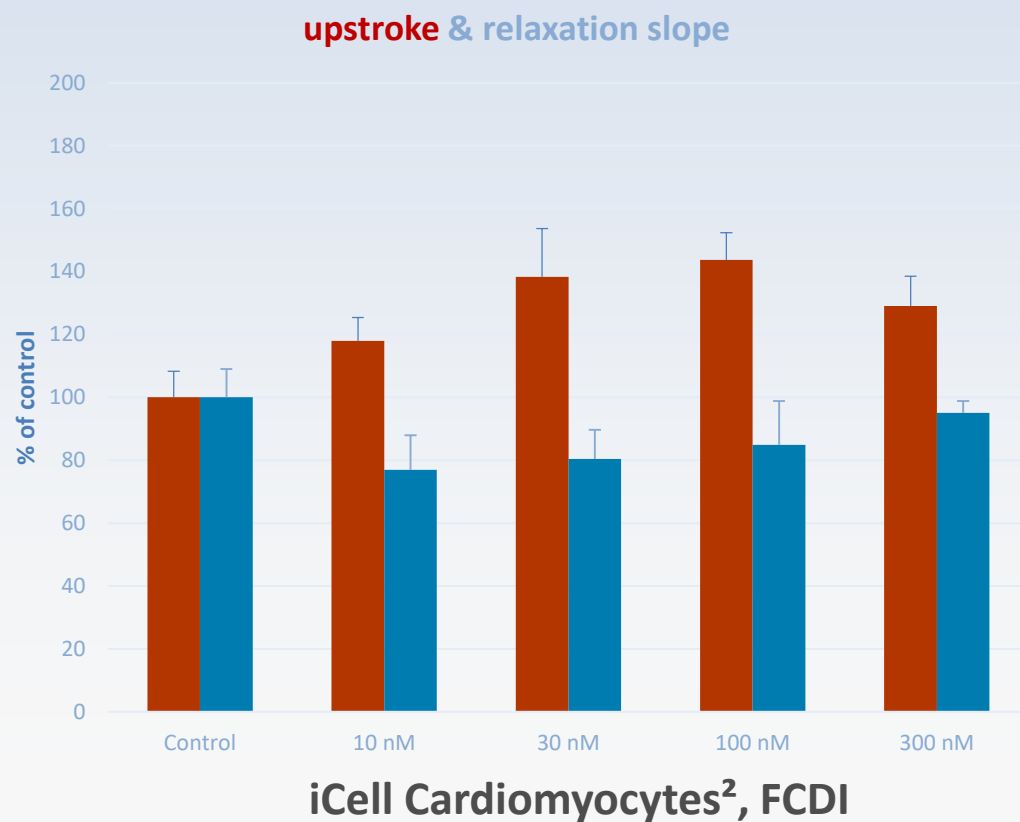


Isoproterenol

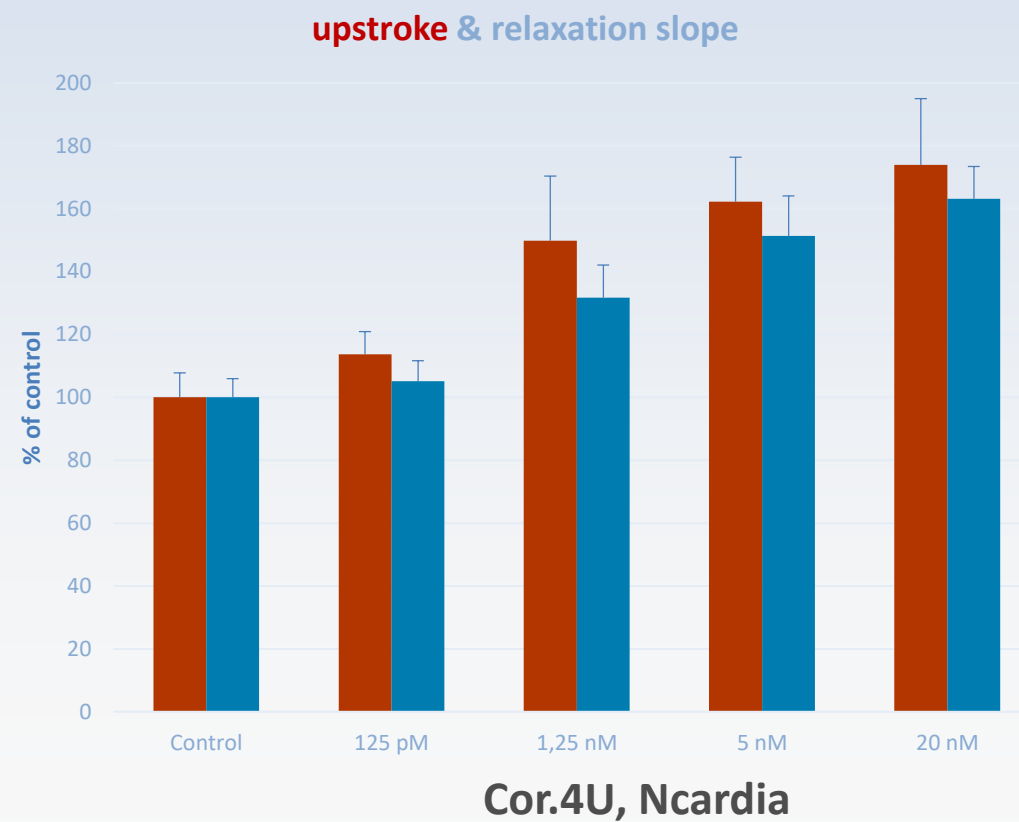


Pharmacology: Positive Inotropes

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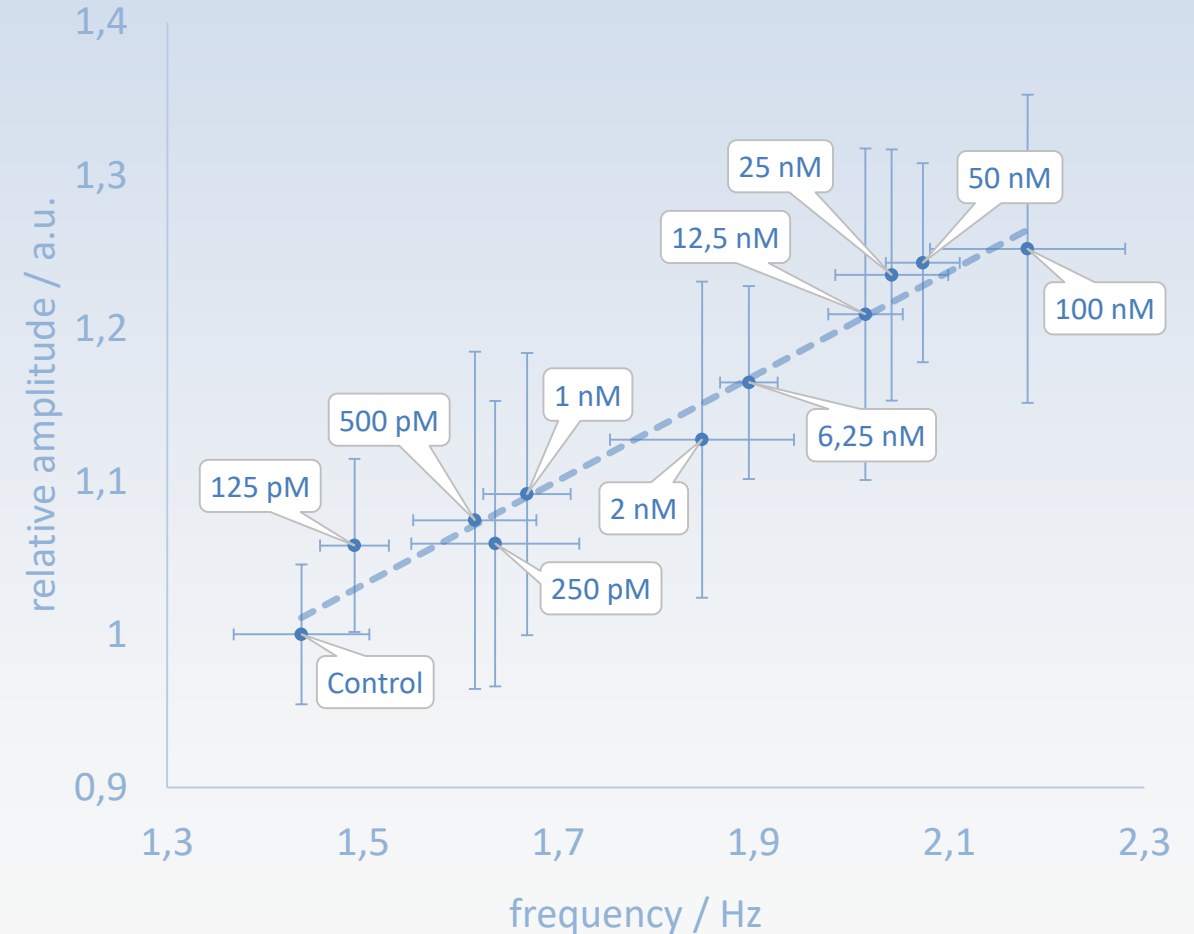


Isoproterenol

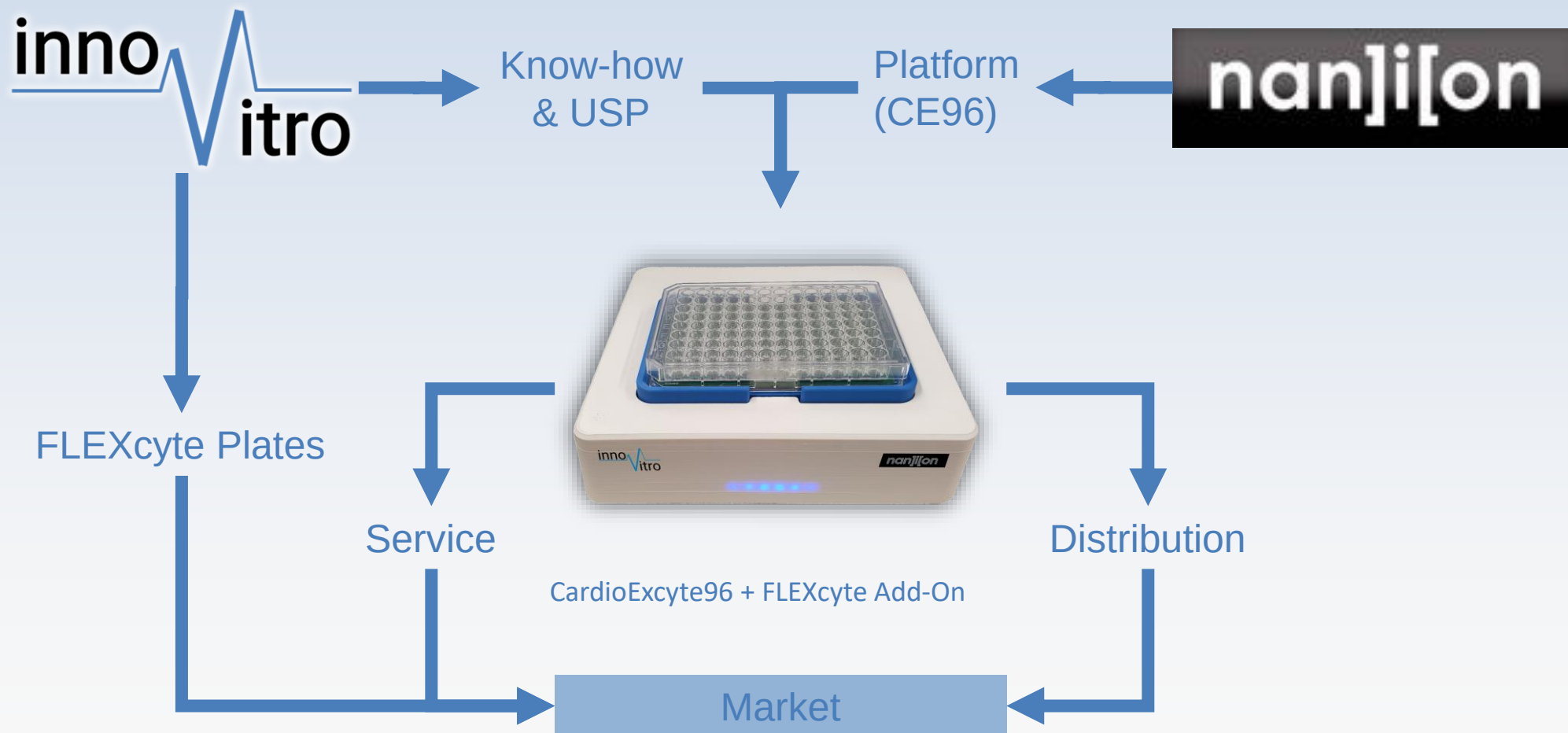


Pharmacology: Positive Inotropes

- Positive force-frequency-relationship with isoproterenol
- Standard culture medium
- 7 days post seeding



The FLEXcyte 96 System



Conclusion

- The FLEXcyte 96 system provides
 - Physiological relevance
 - Precision
 - Throughput
- Integrates into Nanion's accessories:
 - Temperature control
 - CO2 incubation
 - SOL head (optical pacing)
 - etc.

