# Studying Inotropic Compound Effects in Human iPSC-derived Cardiomyocytes using 2D and 3D Models

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## Abstract

The validity of hiPSC-CMs in contractility assays for the evaluation of inotropic drug effects remains challenging. The objective of this study was to measure inotropic responses of hiPSC-CMs using 2D models (impedance and calcium transient) and the 3D human engineered heart tissue (EHT) model (UKE Hamburg), which is currently being validated within the NC3Rs InPulse CrackIT Challenge.

A negative inotropic effect of nifedipine was demonstrated by a decrease in calcium flux amplitude and impedance peak amplitude. Bay K8644 induced an increase in peak width and peak amplitude in both calcium transients and impedance traces. Besides a positive chronotropic effect, isoproterenol induced positive inotropy in the impedance recordings and a tendency of an increase in calcium transients. All expected positive or negative inotropic effects of the compounds were detected with the Pluricyte® Cardiomyocytes in the contraction analysis of the EHT model.

Based on the results we conclude that Pluricyte® Cardiomyocytes were capable of capturing positive and negative inotropic effects, suggesting a relatively mature state of the cells. The ability to quantify contractile force with the EHT model provides an additional powerful tool in the detection of potential inotropic effects of novel drug candidates in a physiologically relevant context.

## Human Pluricyte® Cardiomyocytes are fully functional and show a relatively high level of maturity

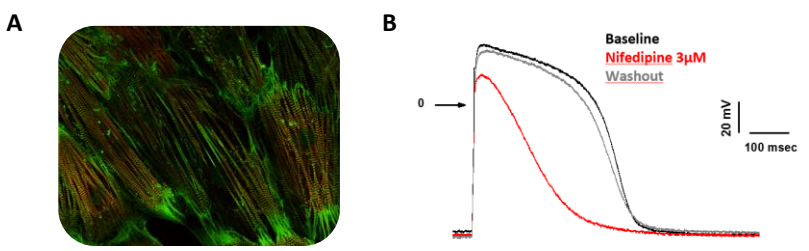
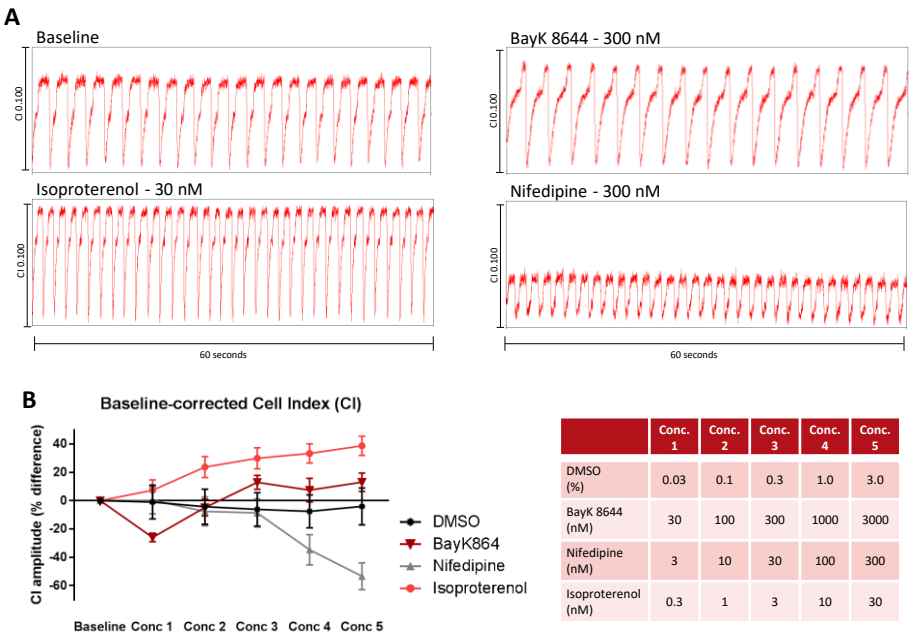



**Figure 1. Characteristics of Pluricyte® Cardiomyocytes cultured in Pluricyte® Cardiomyocyte Medium.**
**A:** Pluricyte® Cardiomyocytes exhibit a high degree of ultra-structural sarcomere organization as determined by immunofluorescence (green: α actinin; Red: myosin heavy chain 7).
**B:** A typical action potential of Pluricyte® Cardiomyocytes (measured by PhysioStim, Lautrec, France), demonstrating a low resting membrane (~-78mV) potential and fast upstroke velocity. As expected, L-type calcium channel blocker nifedipine decreased the APD20 (65%), APD90 (46%) and APA (6.2%).

## Assessment of inotropic compound effects in Pluricyte® Cardiomyocytes using 2D impedance technology




| | Conc. 1 | Conc. 2 | Conc. 3 | Conc. 4 | Conc. 5 |
|---|---|---|---|---|---|
| DMSO (%) | 0.03 | 0.1 | 0.3 | 1.0 | 3.0 |
| BayK 8644 (nM) | 30 | 100 | 300 | 1000 | 3000 |
| Nifedipine (nM) | 3 | 10 | 30 | 100 | 300 |
| Isoproterenol (nM) | 0.3 | 1 | 3 | 10 | 30 |

**Figure 2. Inotropic compound effects of isoproterenol, BayK 8644 and nifedipine on the impedance of Pluricyte® Cardiomyocytes.** Cell Index (i.e. impedance, a surrogate marker for contractility) signals enable assessment of inotropic compound effects. **A:** raw traces of the Cell Index signal show positive (isoproterenol, BayK 8644) and negative (nifedipine) inotropic effects of well-known cardioactive compounds compared to baseline. **B:** Concentration-response curves of the tested compounds. Data were obtained using the xCelligence® RTCA CardioECR instrument.

## Acknowledgments

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Patch clamp data were kindly provided by PhysioStim (Lautrec, France).
Data on the FLIPR Tetra® High-Throughput Cellular Screening System were generated in collaboration with Pivot Park Screening Centre (PPSC).
Mannhardt I[2], Hansen A[2] and Eschenhagen T[2] are co-founders of EHT Technologies GmbH.

## Assessment of inotropic compound effects in Pluricyte® Cardiomyocytes using 2D calcium transient technology

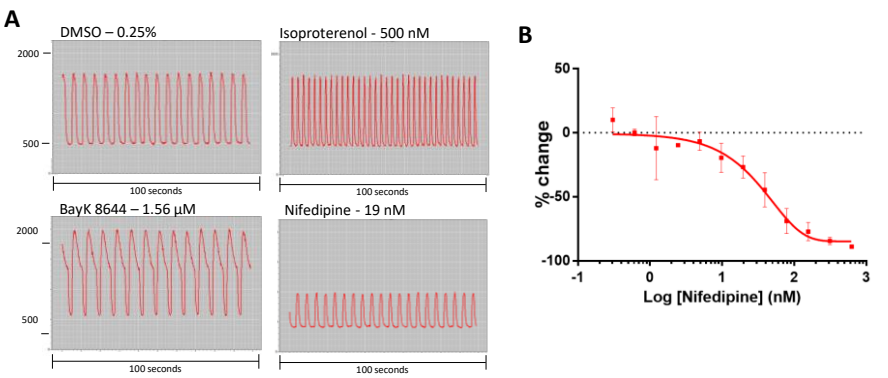


**Figure 3. Compound effects of isoproterenol, BayK 8644 and nifedipine on calcium transients of Pluricyte® Cardiomyocytes. A:** Representative calcium transient signals of Pluricyte® Cardiomyocytes upon compound treatment, showing a positive inotropic effect of BayK 8644, negative inotropic effect of nifedipine and a chronotropic effect of isoproterenol. 0.25% DMSO is shown as vehicle control. **B:** Subsequent concentration-response curves can be used to determine IC50 values of compound effects, such as the negative inotropic effect of nifedipine on calcium transient amplitude (IC50 = 39,52 nM). Data were obtained using the FLIPR Tetra® High-Throughput Cellular Screening System.

## Assessment of inotropic compound effects in Pluricyte® Cardiomyocytes using 3D force generating engineered heart tissues

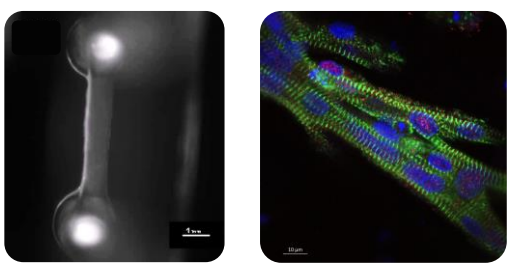

**Figure 4. Engineered heart tissue (EHT) from Pluricyte® Cardiomyocytes**. A fibrin-gel containing dissociated Pluricyte® Cardiomyocytes between elastic PDMS posts in a 24-well format was generated. **Left**: Live image of a 3D, force-generating EHT at day 35 in culture. **Right**: Immunofluorescence staining of cardiomyocytes within the EHT showing elongated sarcomeric structures (green: α actinin, red: MLC2v, blue: DRAQ5).

### Direct contractile force measurement with Pluricyte® Cardiomyocyte EHTs for the assessment of ionotropic compound effects

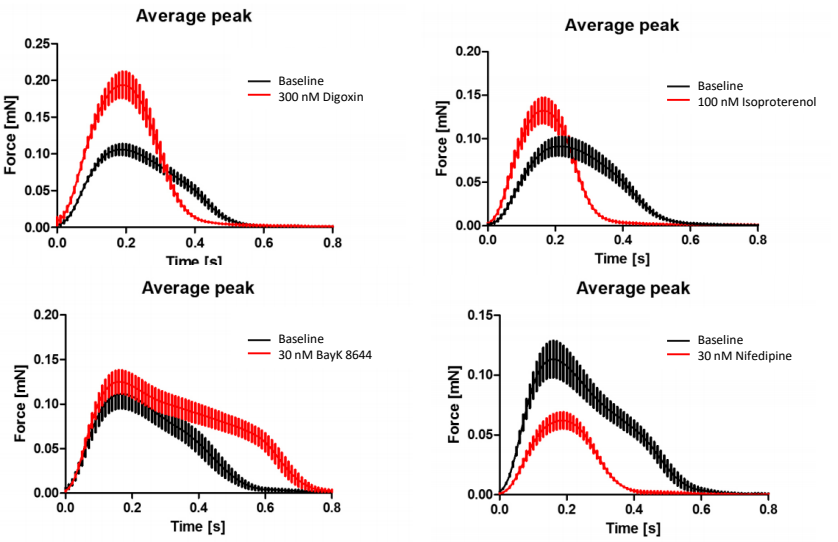


**Figure 6. Inotropic compound effects of digoxin, isoproterenol, BayK 8644 and nifedipine on the contractile force of EHT's from Pluricyte® Cardiomyocytes.** Contractile force of the EHTs show positive (digoxin, isoproterenol, BayK 8644) and negative (nifedipine) inotropic effects of well-known cardioactive compounds compared to baseline. EHT contractions were detected by an automated figure recognition algorithm, and force was calculated based on shortening of the EHT and the elastic propensity and geometry of the PDMS posts.

## Concluding Remarks

- Significant positive and negative inotropic compound effects were detected in 2D and 3D models suggesting the relatively mature phenotype of Pluricyte® Cardiomyocytes. However, a significant positive inotropic effect of isoproterenol was not observed in the calcium transient assay in 2D.
- The ability to quantify contractile force with the EHT model provides an additional powerful tool in the detection of potential inotropic effects of novel drug candidates in a physiologically relevant and more mature context.

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