# Integration of Micropillars in Human iPS-Cell Derived Cardiomyocyte Based Microphysiological Systems for Contraction Force Measurement Enables Precise Monitoring of Pharmacology and Maturation Studies

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**Introduction**: Microfluidic-based organ-on-a-chip devices have the ability to control the cellular microenvironments while enabling real time monitoring of biological processes. The integration of human iPS-cell derived cardiomyocytes (hiPSC-CM) into microphysiological system (MPS) enables the spatio-temporal control of chemical, physical and electrical culture conditions. These can be used to study diseases and develop drugs. In our previous work, we used motion tracking software to monitor cardiac contraction data (beating velocity, rate, direction, prevalence: Mathur *et al. Sci. Rep.* 2015, Huebsch *et al. Tissue Eng. C.* 2015). However, mechanical measurements, such as the contraction force, are also a critical property for a relevant observation of cardiomyocyte maturity and function. Integrating force-sensing micropillars in our MPS system allowed us to derive the force of contraction in a non-invasive, semi-real time, high throughput, and cost-effective way.

**Materials and Methods**: CAD files based mylar masks were created with different micropillar designs of 20um diameter. The cardiac MPS were then microfabricated inhouse using photolithography protocols, PDMS molding and plasma bonding to glass. To form the cardiac microtissue, 20k cells of an 80% hiPSC-CM and 20% stromal cells (hiPSC-SC) solution were loaded in the cell chamber of the cardiac MPS. Physiology and pharmacology were assessed on tissues treated with fatty-acid based Maturation Media (MM) using motion tracking software and fluorescence microscopy. To determine the contraction force, we considered the micropillars as a cantilever beam, vertically fixed on one end and loaded with horizontal forces all the way along the beam. Cardiac cells are wrapping around the pillars and uniformly pulling on the whole length of the pillar. We could then apply the formula of Euler-Bernoulli for uniformly distributed load on cantilever beam and deduct the contraction force from the deflection of the pillar ($\delta_{max}$) upon contraction of the cardiac tissue (Figure 1A). This method was used to perform inotropic studies on the microtissues both in standard and maturation media.

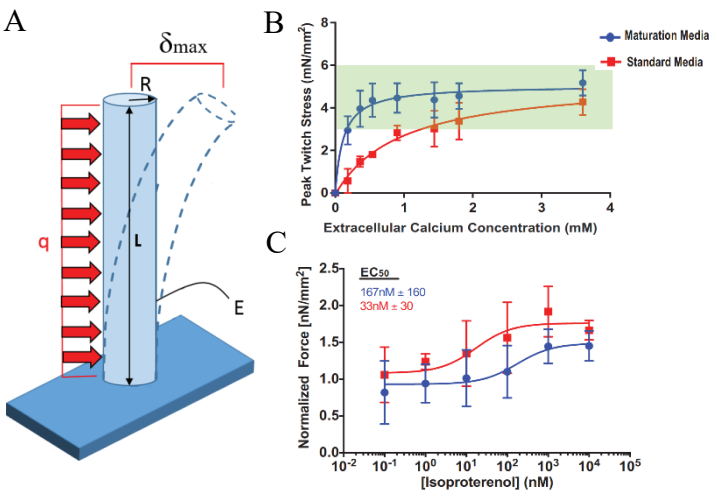


**Figure 1: (A)** Euler-Bernoulli formula for uniformly distributed load on cantilever beam.
**(B)** Inotropic response of either SM or MM pre-treated MPS to the level of extracellular calcium**. (C)** Inotropic response of SM or MM-pretreated MPS to isoproterenol. Data: mean ± SEM, n = 3-5; SM=Standard media; MM=Maturation media.

**Results and Discussion:** Absolute force, normalized to the area of pillar contact, was used to measure the inotropic response of MPS treated with either standard media (SM) or fatty-acid based Maturation Media (MM) to the level of extracellular calcium (delivered in Tyrodde's saline) (Figure 1B). The force was also used to calculate the inotropic response of SM or MM-pretreated MPS to isoproterenol, in Tyrodde's saline containing 0.9mM $Ca^{2+}$ (Figure 1C). These results showed a trend toward increased baseline force and inotropic responsiveness (increase in maximum contraction force over baseline) for MM-pre-treated MPS, although differences between SM and MM pre-treated MPS were not statistically significant. To increase accuracy and throughput, we are developing an automated force measurement algorithm based on Deep Learning technology that would enable the real-time automatic detection of pillars and their movement.

**Conclusion**: We developed an upgraded heart-on-a-chip design with integrated pillar features in the cell chamber. These pillars did not affect the loading nor the survival of the cells, which started beating in a regular fashion throughout the length of the device. We were able to measure contraction force for pharmacology and physiology studies in metabolically matured tissues compared to standard media treated tissues. With the addition of force sensing, our cardiac MPS platform becomes much closer to being suitable for pharmaceutical pre-clinical trials in drug development processes.

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