MICROPOST-BASED FUNCTIONAL ASSAY OF ADULT HEART CELLS: DOES MECHANOSENSING LIMIT FORCE PRODUCTION?

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ABSTRACT

We present a micropost-based technique for measuring axial contractile forces in primary adult cardiomyocytes (PACMs) during the first few hours post isolation from tissue. In typical micropost assays, simple 2-point axial measurements are impossible since cells are suspended across 20-50 posts at a time. This complex loading yields "heat maps" of applied forces instead of force dipoles. Further, current micropost techniques for adult heart cells require 24 hours or more for adhesion, which prevents acute study of PACM function. With our approach, we measure 2-point *axial* contractile forces in healthy PACMs within hours of cell isolation. In our preparations, we observe PACM force generation of several hundred nanoNewtons with a resolution of 136 nN.

KEYWORDS: micropost, PDMS, cardiomyocyte, heart cell, contractile force

INTRODUCTION

Force generation assays of adult cardiomyocytes (CMs) are useful to study functional changes due to pathologies such as dilated cardiomyopathy and hypertrophic cardiomyopathy. Such assays are also used to study mutation-based diseases in heart tissue and to screen for effective drug therapies to improve cardiac function. Currently the gold standard for studying the mechanics of isolated PACMs utilizes pairs of carbon fibers (CFs) adhered to single PACMs and yields 2-point axial force data in the 1-10 μN range [1]. However, CFs are a serial technique requiring substantial time and skill for each measurement. Due to these constraints, fewer than 10 PACMs per isolation can be studied.

Micropost-based technologies for studying PACMs have the benefit of enabling highly parallel measurements that overcome the limitations of CFs. Dense force post *arrays* have been used to study cardiomyocytes, but given the difficulty of culturing PACMs, most micropost work has been conducted on embryonic [2], and neonatal [3,4] CMs. Force generation of PACMs from Wistar rats was previously studied after 1-7 days of culture on force post *arrays, these studies reported per post* forces of around 50 nN/post [5,6]. However, acute measurements were not made because these studies required 1-7 days of culture to obtain firm adhesion before extracting forces. This timing poses a problem because, in contrast to primary neonatal or stem cell derived CMs, which are readily sustained in chronic cultures, PACMs begin to dedifferentiate and remodel in response to their new environment within 24 hours. Further, comparisons of micropost *array* data with CF experiments are problematic because array experiments provide a "heat maps" of force per post data rather than force dipoles. Here, by enabling acute measurements of primary adult myocytes, we report the unique ability to compare the not only PACM disease models but also functionality of CMs at different times of development.

We present a micropost-based technique for measuring axial contractile forces in PACMs during the first few hours post isolation from heart issue. Using a sacrificial-layer technique [3] to study immature cardiac myocytes, we previously enabled the suspension of individual neonatal and stem cell derived cardiomyocytes across pairs of wide-spread micropost sensors. Here, we use this technique without a sacrificial layer to assess acute axial force generation of PACMs within hours of cell isolation. Our approach provides the potential for parallel 2-point axial force generation measurements at a cellular level, which can ultimately be related to tissue level muscle strips. Since our loading arrangement is analogous to 2-point CF measurements, our results can be directly compared with other PACM data obtained using that method.

THEORY

For these studies we assume that PACMs lie suspended across pairs of microposts and that cell sagging is minimal due to the persistent shape of adult isolated CMs. Further, since PACMs shorten about 5-10% (5-10 μm) per contraction, we can assume that micropost deflection is small and therefore that the micropost stiffness is constant in this linear regime. During each contraction the PACM will apply an inward contractile force dipole causing the tops of each micropost to deflect. The magnitude of this dipole is reported as the contractile force. Given this simple loading strategy, the applied contractile force can be calculated as the product of the micropost stiffness, k, and the optical measurement of micropost top deflection, Δx:

$$F = k\Delta x \quad (1)$$

EXPERIMENTAL

Micropost arrays were fabricated via polymethyldisiloxane (PDMS) replica molding of an SU-8 master array as shown in Figure 1, this process is adapted from Tan et al. [7] and is modified with a sacrifical layer technique as previously described

[3]. Posts are 20 μm in diameter and up to 85 μm in height with 60 μm spacing. The effective micropost stiffness was determined via calibration with a piezoresistive cantilever to improve measurement accuracy [4].

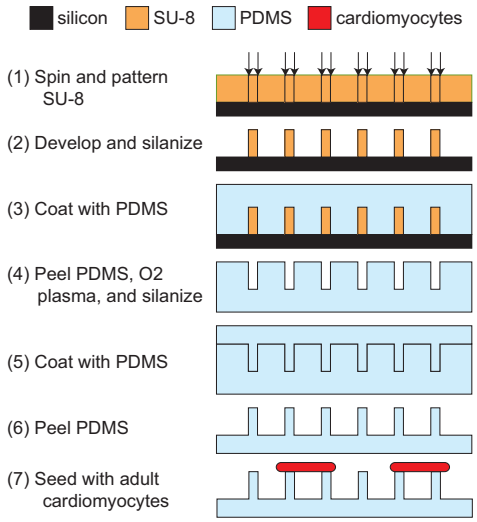

Figure 1: Fabrication and cell seeding process [3]

The devices were O_2 plasma treated and incubated for one hour at 37°C in 10 μg/mL laminin. PACMs from adult Sprague-Dawley rats and FVB mice were cultured as in [8] with two modifications to the protocol: (1) isolated cells were seeded on devices in the plating media and incubated for one hour at 37°C and 2% CO2 to form adhesions, and (2) media was thereafter changed to seeding media and cells were incubated for an additional 20 minutes before measurements were made. During testing PACMs were paced using the commercial Myopacer (IonOptix) system at 1 Hz with a 10 ms biphasic pulse to promote beating. Micropost deflections were optically detected on an upright Leica DMI6000B microscope using a 40X objective and Orca R2 camera.

RESULTS AND DISCUSSION

Figures 2 and 3 present sample data from beating PACMs and corresponding measurements of contractile force. Posts were calibrated and found to have an average stiffness of 840 N/m +/- 70 [4]. We observe contractile forces between 400 to 680 nN in a healthy murine PACM. This force range is similar to measurements of neonatal rat CMs using our method, but less than the micronewton level forces reported for PACMs using CF methods (see Table 1 for summary of contractile force data from micropost-based studies). Surprisingly, dedifferentiating PACMs (as evidenced by Figure 3) may produce higher peak forces but are much more erratic in their force generation and response to pacing.

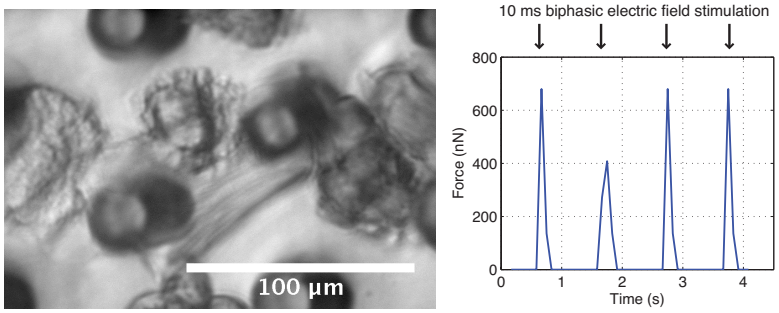

Figure 2: A healthy adult murine cardiomyocyte is suspended between pairs of posts (left), and its contractile forces during pacing are shown (right).

CONCLUSION

We have successfully demonstrated acute measurements of PACMs using a micropost-based 2-point assay. We observed forces of 400 to 680 nN in a healthy PACM while forces in a dedifferentiating PACM showed much higher variability and peak forces exceeding 1 μN. The reported method enables the first acute 2-point axial measurements of PACMs using a force post sensing platform. Our initial peak force data for healthy PACMs are slightly lower than micronewton scale values reported using the CF technique. However, future studies with this platform will explore how peak forces depend on micropost material properties, functionalization protein, and adhesion area. For example, the Frank-Starling relationship for heart

muscle suggests that force generation increases with preload; thus stiffer posts and wider spacing should induce larger contractile forces. Our platform enables these studies for the first time and further measurements are underway to vary micropost stiffness and spacing while retaining constant adhesion areas.

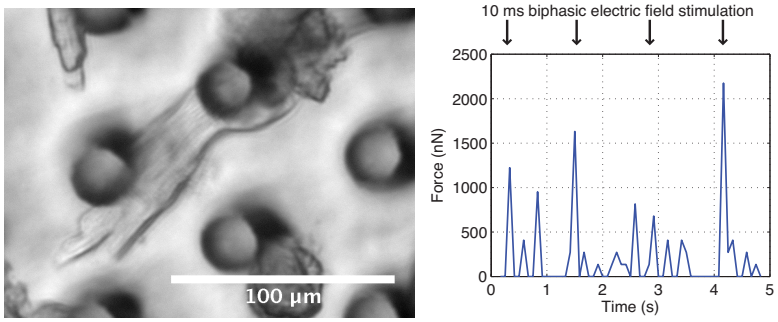

Figure 3: A de-differentiating adult murine cardiomyocyte is suspended between pairs of posts (left), and its erratic contractile forces during pacing are shown (right).

Table 1. Forces and micropost details of previous cardiomyocyte experiments

Ref. Num.	**Previous Micropost Experiment Details**			
	Cell type	*Post bending stiffness*	*Post diameter*	*Force/post*
[3]	neonatal rat	47 nN/μm	20 μm	24 nN
[4]	neonatal rat	60 nN/μm	5 μm	39 nN
[5]	adult rat	293 nN/μm	2 μm	48 nN
[6]	adult rat	~33 nN/μm	~2 μm	100 nN
present work	adult mouse	840 nN/μm	20 um	680 nN

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