BIOMECHANICAL MODULATION OF CALCIUM EVENT RATES IN SOFT MATTER NEURO PATTERNS

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ABSTRACT

Growing neurons in organized patterns has become essential to replicate the complexity of the brain tissue architecture in a dish. A critical feature of a cross-sectional human cortical brain tissue is the tangential curvature, which imposes biomechanical forces due to its bending. How these micropatterns interfere with neuronal signaling, e.g., calcium (Ca^{2+}), remains widely unknown. Here, we present a soft matter-based, low-cost, and multi-scale curvature pattern to study Ca^{2+} events in primary neuronal networks. The resulting neuro patterns allowed us to modulate Ca^{2+} event rates through different curvatures, improving next-generation brain-electrode-interfaces and neurotherapeutic assays.

KEYWORDS: Hydrogel Micropatterning, Curvature Modulation, Primary Neurons, Calcium Communication

INTRODUCTION

In neurobiology and pharmaceutical brain cell assays, neurons are grown in Petri dishes to establish neural networks and connections, dissociated from their complex tissue architecture. The dissociated cells, however, do not regrow networks into their native organization based on densely packed cell layers, spatial growth confinements, and biomolecular gradients but form somewhat randomly organized neuronal network patterns. Through imposing microstructures such as microchannels, adhesion patterns, and cell traps, we can gain some control of the neurite growth pattern [1]. In this context, a curvature-inspired neural network pattern can mimic the architecture of brain folds with larger and smaller curvatures in the sulci and gyri [2]. However, how the imposed microstructures interfere with neuronal communication and subcellular signaling, e.g., calcium (Ca^{2+}) signaling, remains largely unknown. Here, we employed a soft matter-based, low-cost, and reproducible curvature growth technique to build curvature-inspired neural networks (Fig. 1a and 1b). The multi-curvature neuro patterns are achieved through high-viscous agarose hydrogel embossing [3, 4] to prevent cellular adhesion and neurite growth through the hydrogel barrier. We then utilize transient Ca^{2+} signaling analysis to better understand the force impact of multi-curvature bends in patterned versus random-grown neural networks.

EXPERIMENTAL

The neuro patterns are achieved through photolithography, polydimethylsiloxane-based (PDMS) hydrogel embossing following our recently published methodology [5]. Briefly, a KMPR master mold is fabricated on a silicon wafer (height: 50 µm) and then used to cast the PDMS stamp (10:1). For hydrogel embossing, a mixture of type VII-A and I-A agarose was dissolved in phosphate buffer saline and heated to 80 °C. The agarose solution was pipetted onto poly-d-lysine (PDL) coated Petri dishes. The PDMS stamp was then pressed onto the agarose allowing for temperature-based crosslinking (15 min). Rat embryonic brain tissues (E18) were dissected following a previously established protocol [6], and the cortical cells were seeded at a concentration of 250,000 cells/ml onto the soft matter patterns. Calcium signaling was measured using Fluo-4 AM dye with probenecid in the neurite network at eight-day *in vitro* (DIV, 2 fps). Differential interference contrast (DIC) images were captured at 12 DIV to observe network formation. Ca^{2+} signals were recorded (Fig. 1c1, 1 min) and processed using in-house developed software. Regions of interest (ROIs) within distinct curvatures were detected and binned into the corresponding curvature range (Fig. 1c2). Next, signal differences were tracked in each ROI, and calcium events were detected when the signal surpassed a 4-standard deviation threshold (Fig. 1c3-c4). Using equation 1, we estimated normal tensional forces ($|F|$) causing neurite bending, based on the curvature (k) in each pattern, the elastic modulus of neurites (E_N), the average thickness of a neurite (d_N), and the average neurite length L_N.

$$|F| = 4E_N k (L_N)^2 d_N \quad (1)$$

Reference values for each parameters were approximated as E_N = 0.2 kPa (cortical)[7], d_N = 0.5 µm, L_N = 100 µm, and k = 0.004 … 0.03 µm^{-1}.

RESULTS AND DISCUSSION

Agarose-based microchannels on PDL-coated polystyrene surfaces remained intact over a two-week duration in culture. A 2:1 ratio of VII-A and I-A agarose created an effective mixture for imprinting the positive PDMS features onto the PDL-coated dish. Seeded cortical neurons adhered between the soft matter hydrogel structures and grew neurites in circular patterns over nine days *in vitro* (Fig. 1b). We then observed the average Ca^{2+} event rate for each cell at the approximated bending force up to 140 nN exerted on the cell. Randomized network growth yielded 1.27±1.07 Ca^{2+} events/min (mean ± standard deviation). Furthermore, Ca^{2+} event rates in neurite networks grown under low bending forces from 0 nN to 20 nN and from 20 nN to 40 nN were 1.06±0.65 events/min and 1.34±0.93 events/min, respectively and were not significantly different than the rates in randomly-grown networks. Above 40 nN, however, the event rate dropped significantly to 0.74±0.65 events/min. It then continuously decreased for higher force ranges, suggesting Ca^{2+} event silencing at high curvatures.

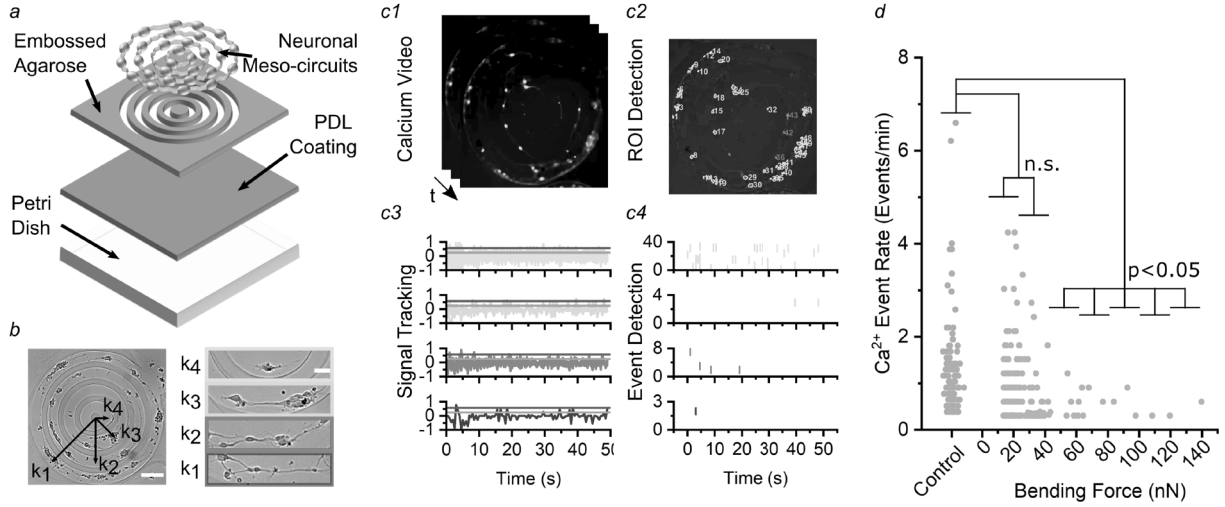

Figure 1. Neuronal cell growth and signaling in a soft matter-based, low-cost, multi-scale curvature pattern. a) Schematic shows the pattern and material assembly. b) DIC image of primary cortical neurons (E18, rat) grown for 12 DIV within the multi-scale curvature pattern (k_1 being the lowest curvature and k_4 the highest). Scale bar = 100 µm (left), 25 µm (right). c1-c4) Image processing workflow for extracting calcium event rates from (c1) image stacks based on (c2) single-cell ROIs, (c3) Fluo-4 AM calcium signal yielding (c4) Ca^{2+} events. d) Calcium event rate distribution across distinct bending forces. (200 µN force increments, significant levels are shown based on Mann-Whitney U Test)

CONCLUSION

Implementing soft matter-based, low-cost, and multi-scale curvature-based neurite network growth into neuronal cell assays allows us to study Ca^{2+} signaling events under a variety of bending forces. This approach provides important insight into the influence of micropatterning features on cellular signaling and tissue engineering and can be further harvested to improve the brain-electrode-interface and neurotherapeutic cell assays.

ACKNOWLEDGMENTS

This work is supported by a National Science Foundation (NSF) CAREER award (A.K., Grant# CBET-1846271). The authors also thank the Montana Nanotechnology Facility at MSU, a member of the National Nanotechnology Coordinated Infrastructure (NNCI), supported by the NSF (Grant# ECCS-1542210).

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