

MICRO MAGNET CHIPS TO STUDY NANOPARTICLE FORCE-INDUCED NEURAL CELL MIGRATION

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ABSTRACT

Mechanical forces have long been studied in relation to stem cell differentiation or cell migration, however, their significance during brain and neural circuit formation are poorly understood. We present a magnetic gradient platform that allows investigation of small scale forces acting on arrays of neurons in parallel. The approach draws magnetic nanoparticles within neurons towards patterned magnetic elements leading to forces acting on neural cell membranes. Initial results show increased cell accumulation and tau activation in neurons adjacent to large-sized magnetic elements. Using our platform provides the possibility to study the impact of force during normal and abnormal brain development.

KEYWORDS: Magnetic gradient chip, Magnetic nanoparticles, Primary neurons, Force-induced cell migration

INTRODUCTION

Neurite regeneration after trauma or brain injuries is still a major hurdle to re-establish functional brain circuits. Reconnecting an isolated neuron to its neighboring cells requires (1) mechanical deformation of the neural cell body (soma), (2) neurite outgrowth, (3) cell polarization and (4) formation of functional synaptic units [1]. To control and initiate these events we need to better understand how neurons form neurite networks and what role biomechanical forces play.

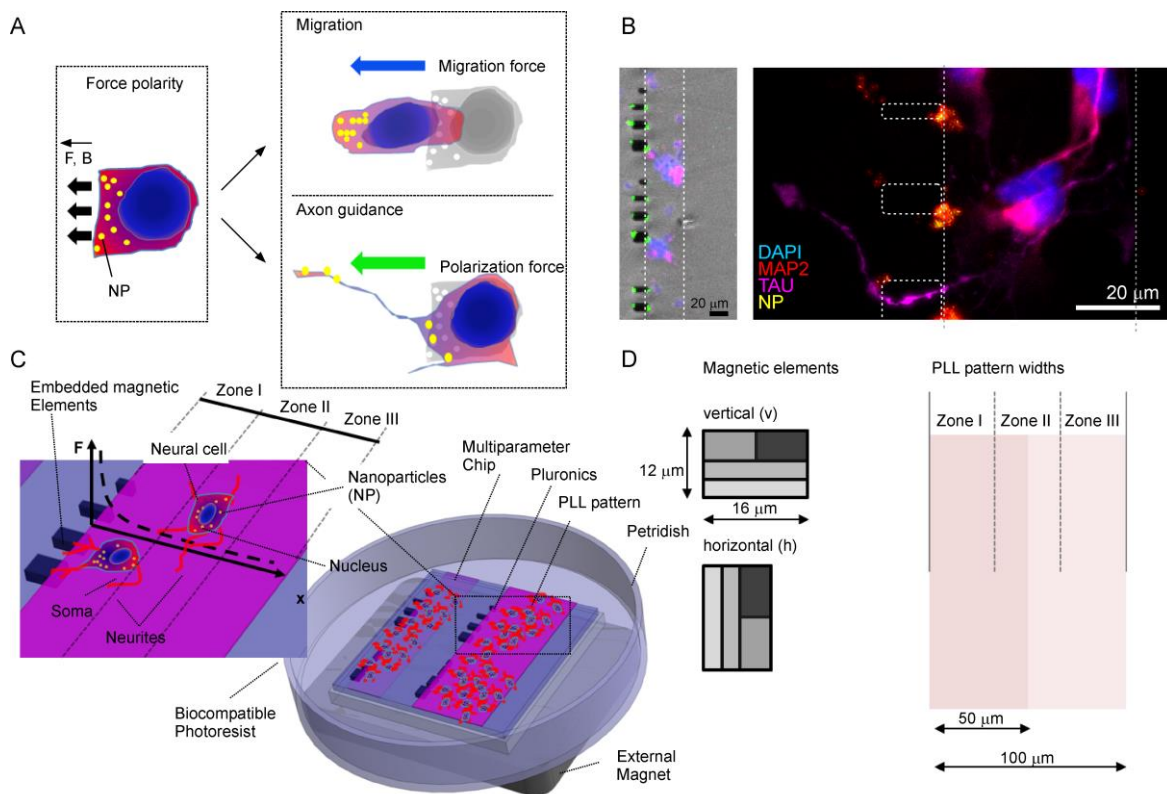


Figure 1: Chip for neuro-magnetic guidance. (A) Applying magnetic nanoparticle induced polar force on primary neurons may allow directional control of neural cell migration and cell polarization. (B) Microchip technology allows highly parallelized multi-parameter analysis to study how neural cells respond to mechanical forces. Microscope image of neurons adhered to PLL patterns next to magnetic elements at a large and small scale. Fluorescent image shows neurons projecting axons (tau: magenta) towards off pattern areas near magnetized magnetic elements. PLL patterns and magnetic element boundaries are highlighted with dashed lines. (NP: nanoparticles, green-yellow, DAPI: cell nucleus, blue, MAP2: dendrites, red). (C) Microscale engineered neural culture chip combines multi-sized cell adhesion patterns with multi-sized magnetic elements generating areas of parallelized magnetic gradients. Zoomed view of neural cell response to intracellular local-induced mechanical forces. Larger magnetic forces (Zone I) are generated through the local amplification of the magnetic field due to embedded ferromagnetic elements. (D) Parameter map shows the dimensions of the magnetic elements and cell adhesion patterns.

This abstract presents a novel magnetic guidance chip to study biomechanical responses in primary neuron cells to locally induced intracellular forces, which may play a role in regenerating neurite networks after injury or trauma. Initial studies investigating mechanical forces on neurons have been limited, investigating few cells at a time, and without significant quantitative data [2]. We adapted a novel micro magnetic chip previously used to localize cell-internalized nanoparticles within arrays of patterned cells [3] to investigate neural cell response to nanoparticle mediated intracellular induced forces in the pico to femto newton range (Fig. 1A). Using our chip multiple parameters, such as cell microenvironments (cell adhesion pattern), cell density, or biomechanical forces (zones), can be examined in a highly parallel fashion (Fig. 1B - D).

THEORY

An induced magnetic field generates a force on superparamagnetic particles, moving them towards the highest magnetic potential. This magnetic force is described through equation (1), where V_p is the volume of the magnetic particle, M_{sat} is the magnetic saturation of the particle and $\nabla H(x,y,z)$ is the magnetic gradient at the location x,y,z induced through an external permanent magnetic field H and μ_0 is the magnetic permeability of free space.

$$F = \mu_0 V_p M_{sat} \nabla H(x,y,z) \quad (1)$$

This magnetic force is balanced by a drag force when particles are accelerated in a fluid. Particles are also directed to the surface for gradients induced by embedded ferromagnetic elements. Due to motion near a surface the particle is further slowed down through a wall drag (Faxen's law) [4], which results in a correction in the drag force equation (2), where μ is the medium viscosity, R_p is the particle radius, λ is the correction coefficient and v is the particle velocity.

$$F = 6 \lambda \pi \mu R_p v \quad (2)$$

EXPERIMENTAL

To study force effects in a parallel fashion we designed a chip with a range of magnetic element sizes that locally perturb an applied magnetic field generating local areas of large magnetic gradients on the chip. The chip surface properties were adapted to allow neurons to adhere on arrays of adhesion stripe patterns in alignment with the ferromagnetic elements. Magnetic guidance was achieved through cell attached and internalized functionalized superparamagnetic nanoparticles.

Micro ferromagnetic elements (NiFe) were electroplated on Ti-Cu microstructures and covered with a low fluorescence biocompatible photoresist (PSR) [3]. Force characteristics of magnetic elements were quantified by measuring the displacement dynamics of $\varnothing 1\mu\text{m}$ magnetic beads (Chemicell, 2101) under applied magnetic field gradients (Fig. 2). For local cell adhesion, PSR was plasma treated (30 W, 45 sec) through opened AZ 5214 microstructures and, after AZ 5214 strip-off, immersed in a PLL/Pluronic solution overnight at 37°C [5]. Cortical and hippocampal brain tissues were dissociated with papain, seeded on the chip and cultured over night at 37°C, 5% CO₂. Adhered neurons were incubated with yellow-green fluorescent superparamagnetic nanoparticles (Chemicell, 4415) for cell internalization for 2 h at 37 °C and exposed to a 65 mT permanent magnetic field. After 24h cell cultures were fixed with 4% paraformaldehyde and immunostained against TAU (axonal marker), MAP2 (dendrite marker) and DAPI (cell nucleus).

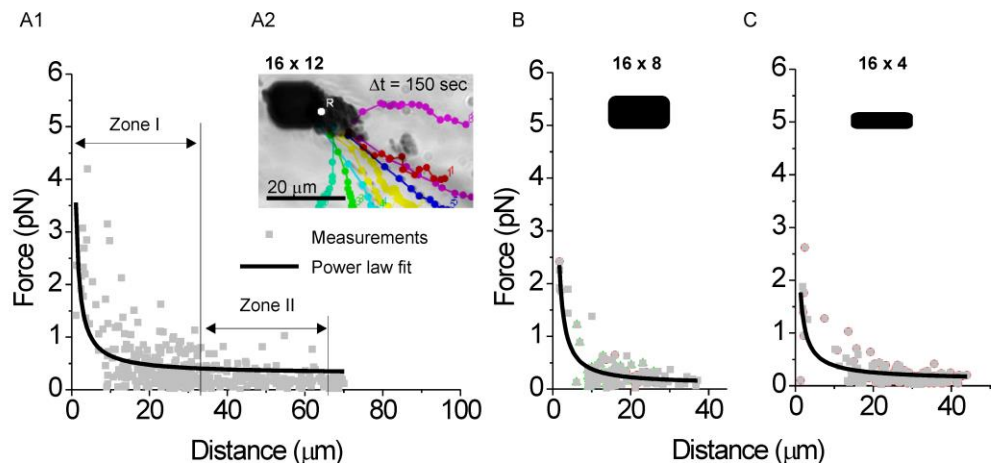


Figure 2: Micro ferromagnetic element induced force gradients were characterized using $\varnothing 1\mu\text{m}$ magnetic microbeads. (A1) Forces were estimated for $12\mu\text{m} \times 16\mu\text{m}$ element from extracted mean velocities and faxen's laws with a correction coefficient $\lambda = 2.29$ [4]. Force distribution was fitted to an analytical power law model. (A2) Extracted video image with overlapped trajectories of attracted magnetic microbeads. (B, C) Extracted force distribution for smaller elements $16\mu\text{m} \times 8\mu\text{m}$ and $16\mu\text{m} \times 4\mu\text{m}$, respectively.

RESULTS AND DISCUSSION

Primary neurons locally adhered to PLL patterns and formed homogenous cultures and neurite outgrowth over two days *in vitro* within the pattern boundaries (Fig. 3 A1). Neurons were positive for MAP2 and TAU staining and showed directed axonal growth over magnetic elements (Fig. 1 B). In contrast, neurons cultured in uniform magnetic fields (no magnetic elements) maintained uniform cell body distribution and uniform MAP2 and TAU expression (Fig 3 A1 and 3 B1). Magnetized magnetic elements attracted free and internalized magnetic nanoparticles, which resulted in a displacement (migration) of neurons towards magnetic elements (Fig. 3 A2 and 3 B2).

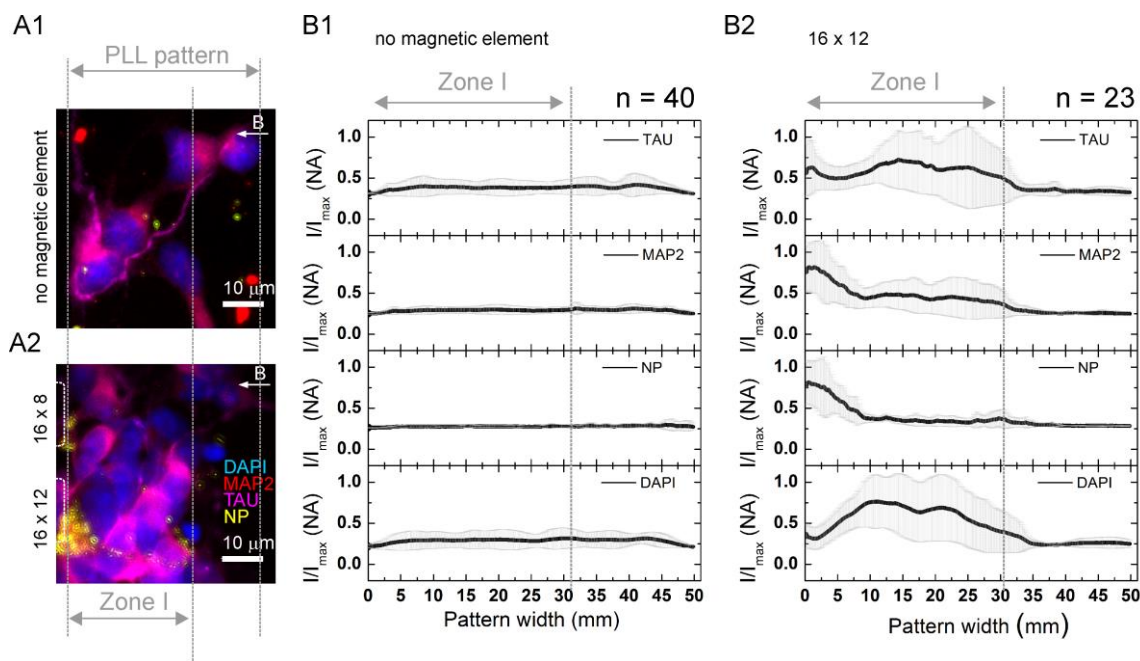


Figure 3: Neurons on PLL patterns in a permanent magnetic field (A1) without and (A2) with magnetic elements. (B) Averaged normalized intensity plots for different neuronal markers(TAU, MAP2), cell nucleus (DAPI) and nanoparticles (NP). $N = 40$ and 23 cells taken from $20 \mu\text{m} \times 50 \mu\text{m}$ ROIs along the pattern or next to the $12 \times 16 \mu\text{m}^2$ ferromagnet respectively.

CONCLUSION

We demonstrated locally-induced neural cell deformation and displacement of neurons (migration) towards the highest strength of magnetic field gradients. Our platform allows us to study multiple combinations of biomechanical forces and how these can direct the organization of neural cell populations in a highly parallelized manner, which can aid in understanding the process of force induced neurite regeneration.

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REFERENCES

1. "Neuronal polarization: The cytoskeleton leads the way", Stuess, M. and F. Bradke, *Developmental Neurobiology*, **71**, 6 (2011).
2. "Biomechanics of single cortical neurons", Bernick, K.B., et al., *Acta Biomaterialia*, **7**, 3 (2011).
3. "Magnetic nanoparticle-mediated massively parallel mechanical modulation of single-cell behavior", Tseng, P., J.W. Judy, and D. Di Carlo, *Nat Meth*, **9**, 11 (2012).
4. "Surface Forces and Drag Coefficients of Microspheres near a Plane Surface Measured with Optical Tweezers", Schaeffer, E., S.F. Norrelykke, and J. Howard, *Langmuir*, **23**, 7 (2007).
5. "Surface chemical patterning for long-term single-cell culture", Cheng, Q., K. Komvopoulos, and S. Li, *Journal of Biomedical Materials Research Part A*, **96A**, 3 (2011).

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