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MICROPOSTS WITH EMBEDDED NANOWIRES TO CONTROL SUBSTRATE STIFFNESS

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Introduction

Cells can sense the stiffness of their substrate and respond through mechanotransduction pathways that lead to changes in cell contractility[1]. Studies to measure the mechanical response of cells have been primarily performed on passive substrates that do not change stiffness as a cell matures. This paper presents a novel substrate that has local regions of higher stiffness. In the current setup, polymer microposts are stiffened with nickel nanowires in random locations. An experiment was run with cells seeded onto the posts which resulted in a promising trend of an increase in cell force per post as the percentage of stiff posts seen by the cell increased.

Methods

Nickel nanowires were created through templated electrodeposition[2]. An alumina filter with 200 nm sized pores was coated with GaIn eutectic and used to create a backing for the wires and placed on a copper electrode in a Watt's nickel plating solution (Fig. 1A-B). The wires were deposited with electrodeposition using a AA battery as a current source. After depositing the wires, the GaIn backing was removed with nitric acid, the alumina filter was dissolved with sodium hydroxide, and the wires were suspended in ethanol after 10 minutes of sonication. The wires and ethanol solution was then seeded onto a PDMS negative mold under a magnetic field to deposit wires into posts as per previous studies[3,4] (Fig. 1C-E). While still under a magnetic field, after the ethanol evaporated, PDMS was poured into the negative mold and heated in a 110 ºC oven for 20 hours.

The random seeding of wires into the posts creates an array of posts that do not have a uniform stiffness. The posts with wires are stiffer than the posts without wires and can be calculated by using Castiliano's Theorem[5], Eq. 1, along with composite beam theory to measure the deflection at the tip of the posts as shown in Fig. 2.

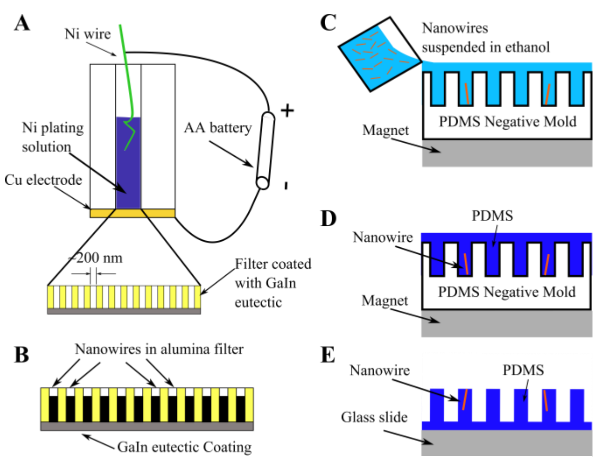

Fig. 1. Nanowires and post fabrication process. A), B), Wires are formed via templated electrodeposition. C) Wires are poured into a PDMS negative mold under a magnetic field followed by (D) PDMS to achieve (E) posts.

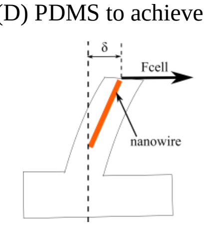

Fig. 2. Deflection of a micropost stiffened by a nanowire.

$$\delta = \frac{\partial}{\partial F}\int_0^L \frac{M^2(x)}{2EI}dx \quad (1)$$

Although it may be off-center or tilted, each wire was assumed to be in a vertical position in the middle of the post. When a wire is at an angle, it may increase the effective stiffness, but the lower bound will be obtained by the equations derived below.

As shown in Fig. 2, the second moment of inertia of the post changes along the length of the post when a wire is contained in the PDMS. This system can be evaluated by the integral shown below in Eq. 2, where E_w is the Young's modulus of the wire, E_P is the Young's modulus of PDMS, L_w is the length of the wire, L is the height of the post, I_w is the second moment of inertia of the wire, and I_p is the second moment of inertia of the post where there is assumed to be a void in the space occupied by the wire. After evaluating the integrals, the effective stiffness of the posts with wires is given by Eq. 3.

$$\delta = \frac{64}{E_p \pi d^4}F\int_0^{L-L_w}(L-x)^2dx + \frac{1}{E_w I_w + E_P I_P}F\int_{L-L_w}^{L}(L-x)^2dx \quad (2)$$

$$k = \left[\frac{64}{3E_P \pi d^4}(L^3 - L_w^3) + \frac{1}{3(E_w I_w + E_p I_p)}L_w^3\right]^{-1} \quad (3)$$

For the experiments in this paper, the posts had a diameter of 2.07 µm and height of 5.62 µm. The nickel wires averaged a diameter or 200 nm and a length of 5 µm. After evaluating Eq. 3, the stiffness of the posts without wires is 38 nN/µm, and the stiffness of the posts with wires is 99 nN/µm. A uniform stiffness change of this magnitude has been shown to cause a change in the force produced by a cell seeded on top of a substrate

A pilot study was performed where 3T3 cells were seeded onto posts stamped with fibronectin and stained with BSA. After the cells adhered to the posts, they were transferred to new media and allowed to spread for 10 hours, then fixed and stained with phalloidin and DAPI and viewed under a microscope. The posts containing nanowires were excluded from analysis as many posts with wires had initial deflections. Posts with nanowires were visually verified by comparing the brightfield image of the posts next to the stained images as can be seen in Fig. 3.

Results

The analysis of traction forces indicated that cells seeded onto substrates with a random seeding of nanowires had a higher average force generation than cells on substrates without nanowires (Fig. 4A). Force data was used for cells on 20-30 posts as force increases with spread area. There were 18 cells studied for no wires, 12 cells studied with <10% wires and 8 cells studied for >10% wires. In addition, by measuring the size of the actin stain, the spread area of the randomly selected cells on the substrate with wires was greater than on the substrate with no wires (Fig. 4B), with 45 no cells on no wires, 22 cells on <10% wires and 19 cells on >10% wires. By analyzing only the wires on the substrate stiffened with wires, the force per post also showed a positive correlation with the percentage of posts stiffened by wires (Fig. 5).

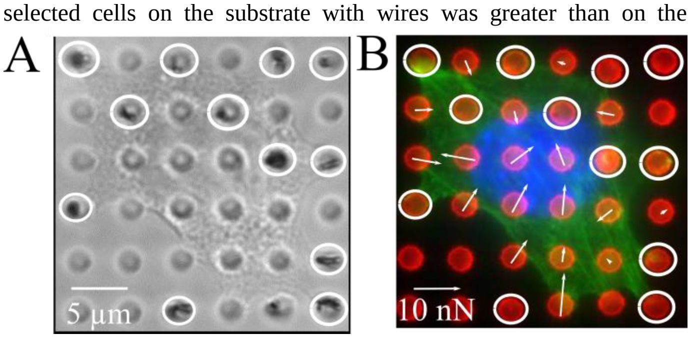

Fig. 3. Circles showing the magnetic posts in the images of a cell. A) Brightfield image containing nanowires. B) Flourescent image with force vectors.

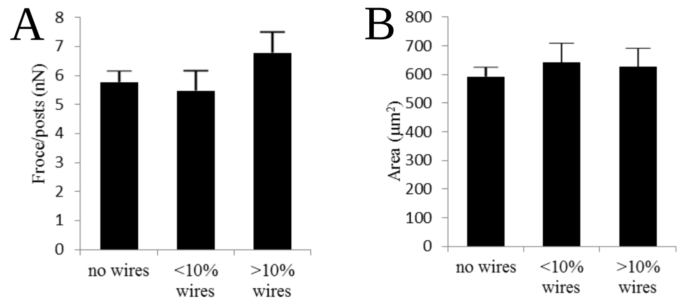

Fig. 4. Microposts stiffened with nanowires cause (A) higher traction forces (p=0.15) and (B) increased cell spreading area, but only for cells seeded on more than 10% magnetic posts. Error bars are standard error.

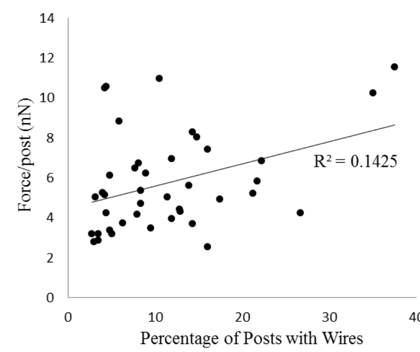

Fig. 5. Positive correlation exists between the percentage of posts with wires and the force per post generated by the cells on top of the arrays.

Discussion

The initial data obtained in these experiments shows a promising trend that as the substrate stiffness increases at discrete points, cells will show an increase in force per post. This work suggests that cells may be able to sense the local stiffness at their focal adhesion and modulate their cytoskeletal tension throughout the structure of the cell. Future work will be to accurately identify the displacements of posts containing wires and to perform experiments to confirm the stiffness of the magnetic posts obtained in Eq. 3.

References

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