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A NOVEL METHOD FOR STUDYING MECHANOTRANSDUCTION: COMPLEX FORCE FREQUENCY APPLICATION USING MAGNETIC VORTEX MICRODISCS

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INTRODUCTION

The current understanding of how cells respond to static and dynamic mechanical forces, particularly as it relates to mechanotransduction, is limited by the lack of an accurate and reliable way to precisely apply mechanical forces using complex frequency profiles in a research environment.

The primary challenge for this type of research lies in the empirical approach that is widely adopted for determining discrete frequencies of applied forces in mechanotransduction studies. This fails to accurately determine the optimal frequencies for force application and is made even less reliable due to the complex nature of cellular motions and intracellular responses to mechanical stimulation.

A method to precisely apply mechanical forces using physiological frequency profiles would deeply enhance the study of mechanotransduction. This would not only provide further tools to grow the fundamental understanding of cellular biology, but also lay a framework for innovative new therapeutic strategies, particularly in diseases where mechanotransduction plays a key role in the pathophysiology [1].

To gain a deeper understanding into the mechanotransduction mechanisms of chondrocytes, we have proposed the use of biofunctionalized magnetic vortex microdiscs for the purpose of applying tensile forces to specific cellular integrins. Magnetic vortex microdiscs, when exposed to an alternating magnetic field, can induce measurable and specific mechanical forces at complex frequency profiles to a single cell. They can provide the specificity needed to appropriately observe changes in cellular behavior such as the catabolic force or frequency thresholds for specific integrins. To accomplish this goal, we will employ the use of a very simple electromagnet built and validated in-house. An example of an application of these disks would be to track $\alpha5\beta1$ and $\alpha1\beta1$ integrins by measuring downstream a myriad of appropriate markers to answer questions such as: How do different levels of static and dynamic tensile forces affect the cellular stress response?

METHODS

It was important to select the optimal design of the magnet system in accordance with the experimental requirements that were posed. The magnet and disk system would be required to apply a controlled force in the range of 5 to 210 pN across the sample. Any magnetic field should be confined to the experimental area and uniform throughout the sample. It was decided that the disks would provide a much higher threshold of control when applying dynamic motion when compared to magnetic beads that have been previously used for similar applications [2].

To properly validate the relationship between the coil voltage (V) and the resulting magnetic field (H), we employed the use of COMSOL for all pre and post construction modeling. This modeling was corroborated using a handheld magnetometer. The force applied to the integrins by the disks will be calculated according to equation 1.

The described process for disk manufacturing involves fabricating magnetic disks (MDs) on a silicon wafer. It begins with the application of photoresist on the wafer, followed by UV exposure through a mask to create a pattern. An organic solvent removes unexposed photoresist. Then, a 5 nm gold underlayer, 60 nm of permalloy, and another 5 nm gold layer are deposited using magnetron sputtering. The MDs are released from the wafer in acetone using a lift-off process. This process enables cost-effective production of uniformly sized MDs with a specific magnetic spin state for use in magnetic storage applications.

The disks are manufactured in conjunction with collaborators from the Center for Nanoscale Materials (CNM) located within Argonne National Laboratory. The final part of disks manufacturing is application of a 5 nm gold coat for the enhancement of biocompatibility and functionalization [3].

Equations

$$(1) \quad F(t) = \frac{2m(t)*H(V,t)}{\pi\beta(t)E}$$

RESULTS

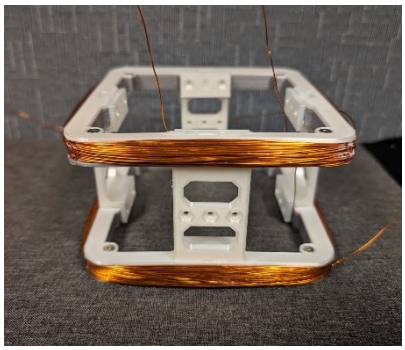

Figure 1: 3D printed Helmholtz Coil

The production and validation of a suitable electromagnet resulted in the development of a small-scale Helmholtz coil that was able to distribute a uniform magnetic field across the sample. The field strength was shown to match the simulation at the lower current that is available without the use of a dedicated benchtop power supply. Upon the arrival of the benchtop power supply, this will be validated again under higher current conditions that still fit the constraints of the wire that is used. Currently, the wire in question is 24 AWG and is rated for use up to 3.5 amps. This amount of current should be suitable to reach past the desired magnetic field strength of 100 Oe.

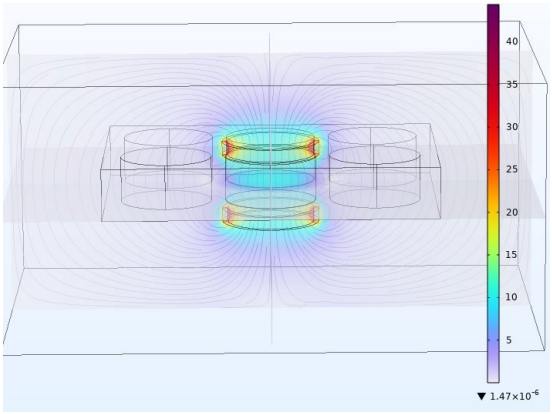

Figure 2: Representative Model of the Electromagnet with a 6-well Plate

The production of wafers during the pre-manufacturing process was completed under direct supervision of experts in the field of nanofabrication at the CNM. Each wafer was observed through optical microscopy to ensure that the shape and size of the disk diameter was ±0.5 µm from the desired size of 3 µm. Through ImageJ analysis, the average disk diameter was found to be 2.903 µm with a standard deviation of 0.048 µm.

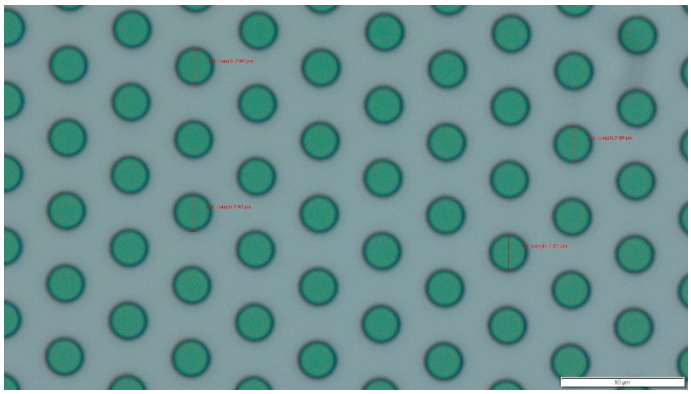

Figure 3: 3 µm Feature Wafer Produced at the Center for Nanoscale Materials

DISCUSSION

Mechanotransduction, the process by which cells sense and respond to mechanical forces, is a fundamental aspect of cellular biology with broad implications for health and disease. However, the current understanding of mechanotransduction is hampered by the lack of precise methods to apply mechanical forces at physiological frequencies in research settings. The commonly utilized empirical approach to selecting frequencies for force application that is commonly used has limitations, as it relies on trial-and-error methods to determine force frequencies, that are almost always discrete in nature. However, that approach fails to recapitulate the complex motions of cells that drive many mechanotransduction processes. The research presented here directly addresses this problem and bridges the existing gap by introducing a new method for applying complex frequency profiles to targeted cell surface mechanoreceptors. The significance of this methodology opens doors to a wide range of mechanotransduction studies in different biological contexts and cell types, which will provide valuable insights into how cells respond to mechanical cues in their native environments. These insights could pave the way for a more complete understanding of disease phenotypes and novel therapeutic options.

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