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BUILDING HOME-MADE OPTICAL TWEEZERS TO STUDY THE MECHANICAL FORCES OF THE CHONDROCYTE CYTOSKELETON IN THE CONTEXT OF OSTEOARTHRITIS

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INTRODUCTION

One way to measure forces in cells is by using optical tweezers. Commercially available optical tweezers are very expensive, costing around $200,000, which makes utilizing this technique inaccessible for many laboratories. Luckily, a low-cost version can be made using parts from a DVD burner. Using the optical pickups of a DVD burner, we can build an optical tweezer assembly that is capable of single cell isolation [1]. In this study, home-made optical tweezers will be built for future use in characterizing integrin motion in chondrocytes for research on osteoarthritis (OA).

OA is a debilitating disease of the cartilage that results in pain, deformity, and loss of function for those who suffer from the disease. OA is the most common form of arthritis and is a major cause of disability in older adults. It is typically referred to as degenerative joint disease, which can be misleading because it is not simply a process of wear and tear, but an abnormal remodeling of joint tissues driven by inflammation. Pathologic changes seen in OA include degradation of cartilage, growth of painful bone spurs, inflammation, and degeneration of ligaments, all leading to joint failure [2]. Surprisingly little is known about the mechanisms that result in the degeneration of the joint that occurs in osteoarthritis.

Chondrocytes are highly specialized, metabolically active cells in articular cartilage that play a major role in the development of OA. These cells work constantly to regulate the surrounding extracellular matrix (ECM) but have a limited potential for replication which contributes to their poor intrinsic healing capacity. Chondrocyte survival depends on an optimal chemical and mechanical environment. Their homeostasis is regulated by mechanotransducers called integrins that respond to mechanical stimulation from the surrounding ECM [3]. In previous experiments integrin motion of chondrocytes was characterized using Atomic Force Microscopy (AFM) force measurement [4]. The AFM experiments on chondrocytes produced exciting results, but there were difficulties differentiating the signal from thermal noise. Because of this we decided to use optical tweezers for validation and to provide more information on chondrocyte integrin motion.

Optical tweezers can be used to directly measure forces that are applied to optically trapped objects. The basic principle behind optical tweezers is the momentum transfer associated with bending light. Light carries momentum that is proportional to its energy in the direction of propagation. Because of this, any change in the direction of light, by either reflection or refraction, will result in a change of the momentum of the light. If an object bends the light, changing its momentum, conservation of momentum requires that the object must undergo an equal and opposite momentum change. This change in momentum is what creates the force that traps the object [5]. By attaching specific molecules to polystyrene microspheres, we can study the forces acting on the molecules under various conditions and use these results to model their physical behavior.

In this study, a home made optical tweezer assembly is built for the future investigation of the movement of the actin cytoskeleton of chondrocytes in the context of OA. The proposed optical tweezer assembly is made from the optical pickups of a DVD-RW drive that is integrated into an inverted microscope with the help of a 3D printed stand and light filters to protect the microscope's camera. The total cost of this assembly is estimated to be about $700. Utilizing this optical pickup build increases the accessibility of this technique by providing a method for optical tweezer experiments at a significantly lower cost.

METHODS

Optical traps behave like linear springs because the trap exerts a linear restoring force on the object. The force of the optical trap can be described as shown in Equation 1, where k is the trap stiffness and Δx is the radial displacement of the bead in the trap. The trap stiffness can be determined experimentally by the application of viscous drag.

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

At low Reynold's number, Stoke's law gives the force on a sphere in a uniform velocity flow field in Equation 2, where η is the viscosity of the fluid, d is the diameter of the microsphere, c is the correction factor for proximity to the glass surface, and V is the velocity of fluid flow [5].

$$F = 3\pi\eta dcV \quad (2)$$

In order to define the trap stiffness, a controlled viscous drag force must be applied to a trapped microsphere. Stoke's law, which describes the motion of spheres through a viscous medium, can be used to determine the applied force. This applied force, along with the experimentally measured displacement of the sphere within the trap, can then be used to determine the trap stiffness.

Using the optical tweezer setup shown in Figure 1, we can measure the basal integrin forces that are present in the chondrocyte cytoskeleton. By functionalizing the beads with fibronectin, we can form a bond between the bead and a chondrocyte that is adhered to a glass surface. By optically trapping the bead and tracking the movement of the bead within the trap we can record the basal forces that are being exerted by the cell on the bead. This information can then be used to validate the results of the AFM experiments and provide more information on the dynamic movement of the chondrocyte cytoskeleton.

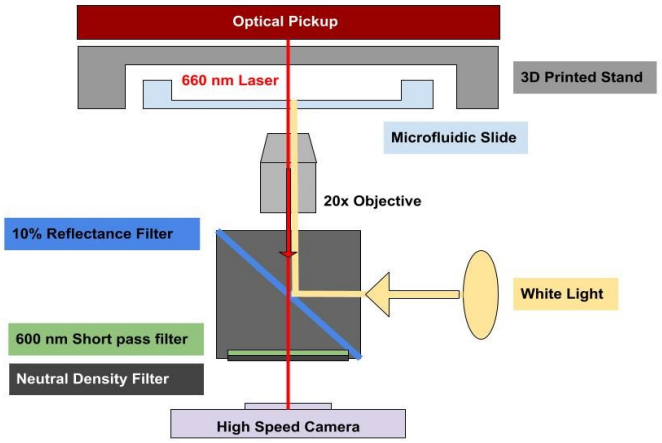

Figure 1: DVD optical tweezer assembly.

RESULTS

To build the optical trap, the optical pickup assembly of a DVD RW drive is integrated into an upright microscope without compromising its imaging modalities. As typically employed, the optical pickup is an electronically steered and stabilized microscope that extracts information from DVD discs. The DVD burner's laser diode is capable of providing the necessary power and focusing optics needed for optically trapping micron-sized particles and cells [1]. The mechanical components from the DVD burner can be used to control the focus of the laser and steer the optical trap to target and capture cells.

The optical pickup assembly used in this project is pictured in Figure 2. Using these optical tweezers, we were able to successfully trap and move a 4 μm glass bead. Unfortunately, soon after trapping the bead the laser diode unexpectedly burned out. Although the laser diode still produced the expected spectrum, the laser power was measured to be less than 10 μW rather than the expected 60 mW, and the laser was no longer capable of forming an optical trap. We initially suspected that the damage was due to electrostatic discharge, as no procedures to prevent this were in place. After sourcing a new optical pickup, we used an antistatic wrist strap when handling the optical pickups to prevent this type of damage, but the measured power of the new laser was still less than 10 μW. Because of this, we plan to investigate the wiring and operating specifications of the pickups as well as exploring other optical pickup sources. Once operational, we plan to measure the optical tweezer's trap strength, as well as the trap strength as a function of voltage. Then, we can begin working on extending its use to cells and developing a protocol for measuring the dynamic motion of the actin cytoskeleton in chondrocytes.

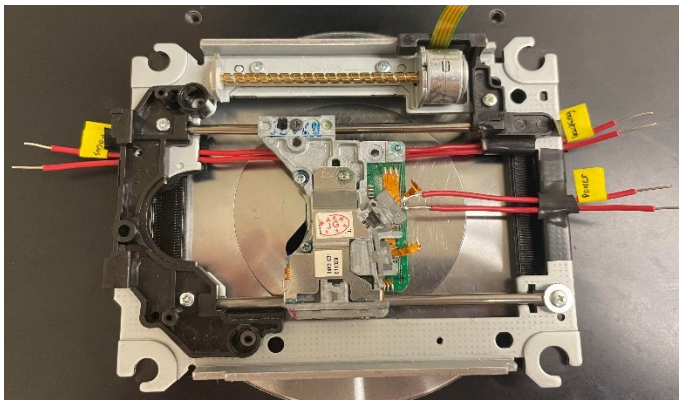

Figure 2: DVD optical pickups.

DISCUSSION

Surprisingly little is known regarding the cellular forces that are present in chondrocytes This project aims to provide a method to unveil the mechanical forces that are necessary for regulating chondrocyte homeostasis. The hypothesis driving this experiment is that the dynamic motion of the actin cytoskeleton, which influences homeostasis in chondrocytes, can be measured using a low-cost optical pickup setup. The project's outcomes will supplement the current understanding of why chondrocytes respond differently to static and dynamic forces. The success of this project will not only provide a path to advance the understanding of OA pathogenesis but also provide a foundation for developing strategies to combat the disease.

OA stands as a leading cause of disability in the developed world, affecting over 32.5 million US adults [6]. Clinical treatments for OA are often limited to pain management and joint replacement due to an incomplete understanding of how this highly complex disease develops. This research will help reveal the intracellular mechanisms for regulation of chondrocyte homeostasis, providing much needed background for advancements in tissue engineering and OA-related healthcare. Additionally, the use of the optical pickup setup for measuring cellular forces in chondrocytes enhances the accessibility of this technique, making optical tweezer experiments more feasible at a significantly lower cost. The affordability of this setup makes it an optimal tool for teaching applications across diverse communities, including those with limited resources.

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REFERENCES

[1] Kasukurti, A. et al., *Optics Express*, 19(11): 10377-10386, 2011.
[2] Loser, R.F. et al., *Arthritis & Rheumatism*, 64(6): 1697-1707, 2012.
[3] Fox, A.J.S. et al., *Sports Health*, 1(6): 461-468, 2009.
[4] Nix, Z. et al., *Prog Biophys Mol Biol*, 176:3-15, 2022.
[5] Brouhard, G.J. et al., *IEEE Trans Biomed Eng*, 50(1):121-5, 2003.
[6] CDC, *Centers for Disease Control and Prevention*, 2020.