Heterogeneous Cell Diagnosis Using Laser Guidance

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Statement of Purpose: The optimal treatment of patients with cancer depends primarily on the ability to diagnosis it in an early stage. Currently cancer is diagnosed using a variety of highly specific histopathological techniques on a patient's tissue biopsy[1]. However, this proves to be difficult in many cases partially due to a lack of specific biological markers. Therefore, there exists a need for a technique that can evaluate a biopsy without the requirement of highly specific cellular markers. Starting from the gene mutation, the development of cancer is accompanied with phenotypical changes at the cellular level including the following: overall size, shape, internal structure, surface membrane properties, etc[2]. Using the force created by a weakly focused laser beam, a phenotypically altered cell can be distinguished from its unaltered form. A change in size or shape results in different effective scattering cross-sections for the beam to interact with, and as a consequence, distinct differences in optical force. Also, changes in the internal structure or surface membrane composition lead to changes in refractive index and, again, changes in the magnitude of force acting on the cell. Therefore, even the most subtle of cellular phenotype changes are distinguishable by evaluating the optical force generated by its interaction with a focused laser source. By simply measuring the traveling velocities of a cell resulting from the optical forces, it is possible to distinguish phenotypically different cell types, ultimately exemplifying a novel and unique method of diagnosing cancer without the need for individual biological markers.

Methods: The laser guidance system, shown in figure 1, consisted of a continuous wave (CW) laser focused by a low numerical aperture lens (NA = 0.27) into a specifically designed guidance chamber. A CW Nd:YLF pumped, Ti:Sapphire laser tuned to 800nm was used to generate the cell guidance. A spatial filter assembly was used in the path of the laser to remove any undesirable noise that can lead to a reduction in beam profile quality. A prism was added to redirect the propagation of the laser vertically prior to entering the focusing lens. This allowed for the optical forces created by the laser to be exerted on a cell in the same axis as that of gravity to remove the variation of the gravitational influence along the guidance path. A CCD camera equipped with a 10X microscope objective was used to image the guidance region within the chamber. These images were exported to specifically developed Matlab code where a variety of functions were used to ultimately determine the traveling velocity of a cell when experiencing the optical force. 3T3 fibroblast and WEHI 164 fibrosarcoma cells were cultured using standard cell culture conditions.

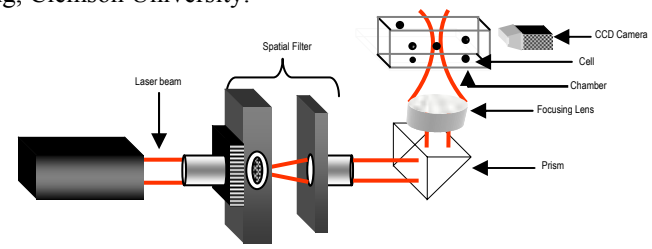

Figure 1. System setup for laser guidance

Results/Discussion: Laser guidance can be used to distinguish one cell phenotype from another. In this study velocity profiles of healthy 3T3 fibroblasts were compared to a cell line fibrosarcoma. Figure 2 shows the data collected from multiple samples of both cell types where their peak velocities, achieved at the focal point of the laser, were compared to one another.

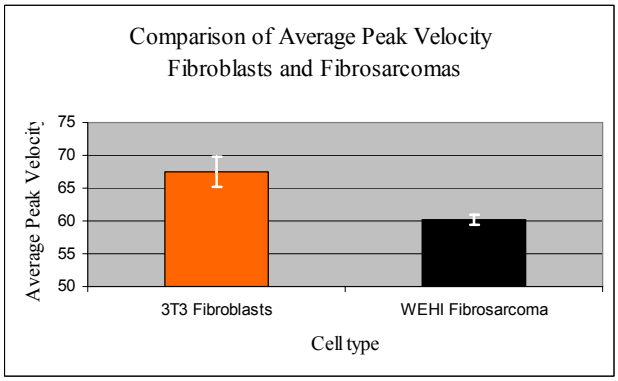

Figure 2. Comparison of peak velocities of fibroblasts and fibrosarcomas in laser guidance

Statistical analysis proved that the average peak velocity of the dissimilar phenotypes were indeed significantly different from one another. Therefore, laser guidance can effectively distinguish changes in a cell's physical characteristics by simply measuring its peak velocity.

Conclusions: A novel system has been developed that has the potential to differentiate phenotypically diverse cells from one another, giving diagnosticians and cell biologists an alternative method for distinguishing between cells, such as cancerous and non-cancerous.

References:

[1] Ramaswamy S. PNAS. 2001;98:15149-15154.
[2] Hanahan D. Cell. 2000;100:57-70.