An Integrated Optical Instrument and Microfluidics for Isolated Chondrocyte, Osteoblast, and Fibroblast Biomechanics

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Introduction: The development, remodeling, and pathogenesis of cartilage, bone and ligaments/tendons depend in part on mechanical signals. Chondrocytes, osteoblasts, and fibroblasts experience multiaxial stress states that maintain the phenotype and production of new tissue. Abnormal mechanical forces alter cell behaviors, resulting in pathological matrix synthesis, increased degradation, and diseases such as osteoarthritis. There is no consensus as to the mechanical signals that are optimally effective in modulating cell function. A pervasive goal is to provide a more complete knowledge of individual cellular biomechanics [1]. The first steps toward this goal have been made with the custom development of an integrated micron-particle image velocimetry and dual optical tweezer (μPIVOT) system. The integrated laser device is capable of quantifying multiaxial biomechanical properties from a single isolated living cell.

As an enhancement of the μPIVOT, microfluidic chips provide additional control of the local microenvironment [2]. The combined optical system with microfluidics establishes a testbed tailored to facilitate flow-based mechanical test sequences including shear and extensional manipulation.

Materials and Methods: The distinct wavelengths of the μPIV and OT lasers facilitate optical integration. A dual OT system was constructed with a 1064nm wavelength laser and infrared optics in order to split/direct the laser into an inverted microscope. The beam reflected off a low pass filter, passed through a 1.4 numerical aperture 60x objective lens, into the cell culture. High resolution force (pN) and displacement (nm) measurements account for both cell and trap stiffnesses [3]. The μPIV system consisted of a 532nm wavelength laser connected with a fiber optic cable into the inverted microscope. The laser pulses were reflected by a high pass filter cube through the objective lens illuminating a culture flow field, seeded with 275nm diameter fluorescent polystyrene spheres. The emitted light (wavelength>550nm) returned through the objective lens, the low and high pass filter cubes, and recorded by a digital camera for image cross-correlation.

Microfluidic chips were designed and fabricated with soft lithography to produce 50μm deep x 500μm wide microchannels. Chondrocytes, osteoblasts, and fibroblasts were cultured from rat cell lines in preparation for biomechanical testing. Mechanical stresses were applied either through direct laser manipulation from the dual OT or through fluid induced stresses from straight or cross-junction microchannel flow fields.

Results: Laser power measurements indicated a 140pN trapping force with 0.3W, typical for biological applications. With μPIV, the velocity profiles were measured in microfluidic chips while successfully trapping polystyrene microspheres (Fig 1). A straight channel flow created a drag force of 82pN (Fig 2). Particle displacement relative to the trap focal point during this flow regime indicated a trap stiffness of 16pN/μm. Localized flow velocity gradients measured with μPIV at the cross-flow resulted in a flow field with shear strain rates of xy=22.07 +/- 1.38 and yx=21.51 +/- 4.62 (strain/s +/- standard deviation)(Fig 3).

Discussion: Ongoing quantification of multiple load regimes will validate the applied optical and fluid stresses, examine elastic-plastic deformations of deformable particles and cells, and explore the biomechanics of normal and pathologic cells. The long-term goal is to provide a more complete knowledge of individual cellular biomechanics and prioritize the biomechanical factors most critical to stimulating regenerative or degenerative pathways.


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