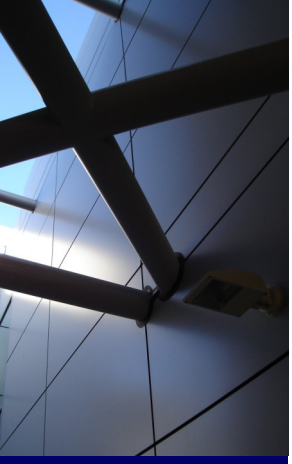

MECHANICAL ENGINEERING

The Department of Mechanical Engineering presents:

The Ph.D. Dissertation Defense of Yanyan Zhang

Tuesday, November 20, 2012
11:00am-1:00pm
Bourns Hall A341

MEMS-based Massively-parallelized Mechanoporation Instrumentation for Ultrahigh Throughput Cellular Manipulation

Doctor of Philosophy, Graduate Program in Mechanical Engineering
University of California, Riverside, December 2012
Dr. Masaru P. Rao, Chairperson

Many applications in cell biology, genetic engineering, cell-based therapeutics, and drug discovery require precise and safe methods for introducing membrane-impermeable molecules into cells. This can be implemented satisfactorily by microinjection. However, disadvantages of traditional manual microinjection include high degree of operator skill, low throughput and labor-intensiveness. Many studies have focused on developing automated and high-throughput systems for microinjection to address these limitations. However, none have provided sufficient throughput for applications such as ex vivo cell therapy, where manipulation of many millions of cells is required. Herein, we propose an ultrahigh throughput (UHT) mechanoporation concept that seeks to address these limitations. The mechanoporation device is a massively-parallelized MEMS-based platform for passively delivering molecules into living cells via mechanical cell membrane penetration. Studies focusing on device design, fabrication and validation at the proof-of-concept level are presented in this dissertation.

Detailed system concept and design is introduced, which integrates functions of cell transfer, capture, penetration and release into a single piece of instrumentation using a microfluidic approach. System operating parameters are analytically analyzed and numerically simulated. Results from these studies agree with previous studies by others in related applications, and suggest reasonable operation feasibility without detrimental effect on cells. Those estimated operating parameters also provide basis to develop test models in practical cell studies. The device fabrication utilized conventional silicon MEMS technologies, and we successfully produced millimeter-scale device chips containing an array of ten thousand hemispherical capture wells with monolithically integrated solid penetrators. A flow circuit system involving a syringe pump, pressure transducer, and fixture set supporting the device chip was developed, and preliminary functional testing was carried out. Device validation tests using K562 cells obtained about 15% average penetration efficiency of live cells after manipulation. Subsequent testing with fluorescent beads and Mouse Embryonic Fibroblast (MEF) cell identified several key issues responsible for the lower-than-expected efficiency, thus suggesting that improved performance may be possible with further system and operation optimization.

The UHT mechanoporation device developed in this effort shows promise for providing an efficient and safe method for introducing membrane impermeable molecules into cells with ultrahigh throughput. Moreover, these studies also represent key steps towards our long-term goal of developing instrumentation capable of UHT cellular manipulation via active microinjection. This new instrumentation will have broad potential for advancing understanding of fundamental cellular processes, as well as facilitating clinical translation of cell-based therapies.